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PULMONARY HEMORRHAGE IN INFANTS
A DESCRIPTIVE STUDY*

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Pulmonary hemorrhage is a common occurrence in infants. Several types are seen grossly, varying from those which cause petechiae to massive hemorrhage. However, few studies have been made on the pathogenesis of pulmonary hemorrhage, except for the petechial type.

Petechial pulmonary hemorrhages were considered by Tardieu[1] in 1855 to be a sign of suffocation. Many subsequent studies have been made,[2-5] and it has been generally believed that such hemorrhages may occur in many diseases and are not specific for any one condition. Walcher[6] found that hemorrhages within the lung occurred as frequently, and were of the same significance, as subpleural hemorrhages. More recently, Clifford[7,8] described small pulmonary hemorrhages associated with asphyxia and stated that these may arise either from diapedesis or by rupture of small blood vessels.

Massive pulmonary hemorrhage in 2 premature infants has been recorded by Ylppö.[9] Browne[10] reported its occurrence in 7 of 80 infants on whom post-mortem examination was performed. To explain this incidence, these authors stressed the significance of infection and weakness of the vessels in newborn infants.

It is our purpose to present a descriptive study of necropsy material, which may help in formulating the pathogenesis of pulmonary hemorrhage in infants, and to describe possibly relevant changes in the structure of the pulmonary veins during fetal and early neonatal life. The following questions have been considered: (1) Is massive pulmonary hemorrhage important as a cause of death in infants? (2) Are different types of pulmonary hemorrhage encountered and are these types correlated with clinical and post-mortem findings? (3) Is there a difference in the incidence and character of hemorrhages in premature, full-term, and stillborn infants? (4) What is the rôle of infection

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in the development of pulmonary hemorrhage? (5) What non-infectious factors are concerned in the pathogenesis of pulmonary hemorrhage?

Three anatomical problems were explored in this study. (1) Is there a structural difference between the intrapulmonary veins of newborn premature and full-term infants? (2) Is there a difference in the rate of development of the intrapulmonary veins in premature and full-term infants during the first month of extra-uterine life? (3) Are there signs of structural weakness in the walls of veins in newborn infants?

Material and Methods

Pulmonary hemorrhage was considered to be massive if it severely involved one-third or more of the lungs, grossly. Four thousand necropsy records from 1927 to 1946, inclusive, were reviewed, revealing a total of 67 cases of massive pulmonary hemorrhage, 37 in boys and 30 in girls. Lung sections from these 67 infants and from 240 unselected infants were studied. Sections of from two to ten paraffin blocks were examined in each case. All were stained with hematoxylin and eosin. In some cases, sections were studied with other stains such as Weigert's or Verhoeff's elastic tissue methods, van Gieson's stain, Mallory's aniline blue, phosphotungstic acid hematoxylin, or Foot's reticulum stain. Hematoxylin and eosin stains were adequate in most instances.

For the studies on vascular structure, lung sections were obtained from infants at the Children's Hospital and the Boston Lying-In Hospital. Paraffin sections were stained with hematoxylin and eosin, aniline blue, Weigert's and Verhoeff's elastic tissue stains, and Foot's reticulum stain. The amount and the character of elastic tissue serve as good criteria in estimating the development of veins, but it is possible, and important, to observe other structural differences by using aniline blue, hematoxylin and eosin, and hematoxylin-van Gieson stains to demonstrate compactness of the inner layer and sharpness of the borderline between the inner and outer layers. Sections of from one to five paraffin blocks were reviewed in each case.

The birth weights of the infants varied from 450 to 4,000 gm. (Table I). Of the 240 infants seen at post-mortem examination, 229 were less than 1 year of age, and 196 less than 1 month. Of the latter, 138 were premature, and, in 79 per cent of these, hemorrhage of some type had occurred. Fifty-eight of the 196 were full-term infants; in 70 per cent of these hemorrhage had occurred. Lung sections obtained at the Boston Lying-In Hospital from 50 unselected, stillborn infants were studied also. In 54 per cent of these hemorrhage had occurred.

TABLE I

Ages of Premature and Full-Term Newborn Infants Grouped According to Weight at Birth

Group	Weight at birth	Stillborn	Age				Total cases
			1 day or less	2 to 6 days	7 to 14 days	15 days to 5 weeks	
	gm.						
1	450– 750	1	1	2	0	1	5
2	751–1,000	1	2	2	2	1	8
3	1,001–1,250	1	1	1	1	2	6
4	1,251–1,500	1	4	1	1	1	8
5	1,501–1,750	1	4	2	1	2	10
6	1,751–2,000	1	2	1	1	2	7
7	2,001–2,500	1	1	1	1	1	5
Full term	2,501–4,000	2	1	4	3	2	12
Total		9	16	14	10	12	61

RESULTS

Conditions Associated with Massive Pulmonary Hemorrhage

Table II shows the ages of 67 infants with massive pulmonary hemorrhage compared with the ages of 2,614 unselected infants examined post mortem. Associated conditions in the 7 patients older than 1 year included leukemia in 3, overwhelming acute infection in one, hemorrhagic pneumonia in 2, and streptococcal septicemia in one. The rôle of infection in these patients will be considered later.

TABLE II

Ages of 67 Infants with Massive Pulmonary Hemorrhage Compared with Ages of 2,614 Unselected Infants Seen at Necropsy *

Age	Unselected infants seen at necropsy		Infants with massive pulmonary hemorrhage	
	Number	Per cent	Number	Per cent
3 days or less	295	11.3	27	40.2
4 to 7 days	154	5.9	15	22.4
8 to 14 days	120	4.6	10	14.9
15 to 29 days	233	8.9	1	1.5
1 month to 1 year	957	36.6	7	10.5
Older than 1 year	855	32.7	7	10.5
Total	2,614	100.0	67	100.0

* Ages of patients were available in only 2,614 of the 4,000 necropsies performed from 1927 to 1946, inclusive.

In 42 cases, massive pulmonary hemorrhage in infants less than 1 year of age was not associated with infection. Sixteen of these infants

were premature and 26 were born at term. Associated findings in these cases are shown in Table III.

Patients who had kernicterus died at from 2 to 8 days of age. Alveolar and septal hemorrhages were seen in 4 of the 10 cases in which there was kernicterus; alveolar hemorrhage alone was seen in 6. In 4 cases there was no evidence of pneumonia microscopically, al-

TABLE III

Findings, Other Than Those of Infection, Associated with Massive Pulmonary Hemorrhage Occurring in 42 Infants Less Than 1 Year Old

Associated finding	Number of infants		
	Full term	Premature	Total
Kernicterus	7	3	10
Congenital heart disease	8	0	8
Postoperative cases	5	0	5
Intracranial hemorrhage	3	10	13
Transfusion	3*	3	6
Total	26	16	42

* One in group of postoperative cases; 2 in group with congenital heart disease.

though in 2 of these there were positive lung cultures. These findings indicate the direct association of kernicterus with pulmonary hemorrhage in the extremely young infant.

The types of congenital heart disease associated with massive pulmonary hemorrhage included coarctation of the aorta with widely patent ductus arteriosus in 2 cases and interventricular septal defect in 6 cases, 4 of which were associated with other malformations. Six of these infants died in the first week, one in the second, and one at 4 weeks of age. In 4 instances infection was present. In one case of coarctation and in one of septal defect, transfusions of 50 and 70 cc., respectively, had been given. Alveolar hemorrhage, probably of capillary origin, and severe congestion were present in all cases. Septal hemorrhage was present in all but one.

Five cases of massive pulmonary hemorrhage occurred in from 9 hours to 8 days after operation in patients from 2 to 10 days old. Operation had been performed for meconium ileus in 2 patients, congenital atresia of the ileum in one, tracheo-esophageal fistula in one, and diaphragmatic hernia in one. Infection was a prominent complicating factor in each instance. In the case of tracheo-esophageal fistula, evidence pointed clearly to an overloading of the circulation with fluid, including blood. The microscopic appearance of the hemorrhage in this case was unusual. Intrapulmonary arteries were sur-

rounded by wide zones of hemorrhage arranged in two layers, the inner one containing hemosiderin (Fig. 1). Perivenous hemorrhage was less distinct, consisting of a single zone without hemosiderin. Alveolar hemorrhage was severe and extensive. Pneumonia was not found microscopically, although *Escherichia coli* and *Streptococcus hemolyticus* were cultured from the heart's blood. Lung cultures were not made.

Considering the large number of operations at the Children's Hospital, the incidence of postoperative massive pulmonary hemorrhage is low. The few cases seen have occurred in patients with severe congenital disease requiring prompt surgical intervention and have been complicated by infection and, in one case, by overloading of the circulation with fluid.

Massive pulmonary hemorrhage was associated with intracranial hemorrhage in 13 infants. It occurred in 3 full-term infants and in 10 premature infants. In full-term infants, intracranial hemorrhage was due to tentorial tear in 2 cases and to generalized massive subdural hemorrhage in one. These 3 infants died before the third day of life. Evidence of infection was present in all 3. In one case the prothrombin time was greater than 3 hours. Septal and alveolar hemorrhages were present in 2 cases and alveolar hemorrhage alone in one. Of the 10 premature infants with intracranial hemorrhage, 6 had intraventricular hemorrhage, 3 had subarachnoid hemorrhage with clots of blood about the base of the brain (one of these also had subependymal hemorrhage), and one had a small subarachnoid hemorrhage. These patients died at from 2 to 10 days of age. Pneumonia was present in 4; 8 showed positive cultures from the blood or lungs and 2 showed no signs of infection. In 2 cases, evidence of severe asphyxia was present. Alveolar and septal hemorrhages occurred in 9 of the premature infants; alveolar hemorrhage alone, associated with overwhelming streptococcal septicemia, occurred in one, the smallest of the premature infants (weight, 675 gm.).

Conditions associated with 14 additional unclassified cases of massive pulmonary hemorrhage in full-term infants included: adrenal hemorrhage with pneumonia in one case, intra-uterine asphyxia in 2, upper respiratory obstruction not due to infection in 2, thrombosis of vena cava, aorta, and renal veins in one, neonatal asphyxia with hypothermia in one, "hemorrhagic disease of the newborn" in 2, epidemic diarrhea in one, botryomycotic abscess of the lung in one, severe, multiple congenital anomalies in one, malnutrition with acute and chronic pancreatitis and arrested physical development in one, and acute nutritional disturbance in one.

Massive Pulmonary Hemorrhage in Premature Infants. Ten cases of intracranial hemorrhage and 3 of kernicterus in premature infants have been referred to previously. Massive pulmonary hemorrhage in premature infants was associated also with the following non-infectious conditions: severe intra-uterine asphyxia in one case, toxemia in the mother in 2 (one with transfusion), "hemorrhagic disease of the newborn" in 2, and transfusion alone in one. The weights of the premature infants varied from 675 gm. to 2,100 gm. Alveolar and septal hemorrhages occurred in 17 premature infants with massive pulmonary hemorrhage. Alveolar hemorrhage alone occurred in one infant who had kernicterus with interstitial pneumonia and bacteremia caused by *Staphylococcus aureus,* in one who had intracranial hemorrhage with overwhelming streptococcal septicemia, and in one to whom transfusion had been given.

Effect of Transfusions on Massive Pulmonary Hemorrhage. In 6 cases of massive pulmonary hemorrhage (3 in full-term and 3 in premature infants), transfusion was believed to be of etiologic importance. In 2 infants (2 and 4 days old, respectively), coarctation of the aorta, of the fetal type, with widely patent ductus arteriosus, was present also. One received 50, the other 70 cc. of blood. In one instance (previously mentioned among the postoperative cases) massive pulmonary hemorrhage occurred in an infant 10 days old who had received 50 and 55 cc. of blood on successive days in addition to 700 to 900 cc. of fluid on each day. The 3 premature infants who received from 50 to 80 cc. of blood each, died at from 2 to 10 days of age. The patients in whom transfusion was thought to be an important cause of massive pulmonary hemorrhage died during or shortly after transfusion or presented some signs of pulmonary hemorrhage, such as blood in the nose, dyspnea, or collapse, during or immediately after transfusion. Although signs of pulmonary hemorrhage were noted early, death occurred as late as 1 day after transfusion. Alveolar and septal hemorrhages were seen microscopically in these infants, with the exception of one who had alveolar hemorrhage only and one who had generalized periarterial hemorrhage (Fig. 1).

Rôle of Infection in Massive Pulmonary Hemorrhage. Because of the common occurrence of infection in these infants and its importance in neonatal pathology, it will be presented separately. To determine its rôle in the production of massive pulmonary hemorrhage, all available sources of information were utilized: clinical history, cultures taken from living patients, post-mortem cultures, and gross and microscopic observations.

No microscopic evidence of infection was present in the 3 infants

older than 1 year who had leukemia, although blood cultures showed *Staph. aureus* and lung cultures showed alpha streptococci in one and *Staph. aureus* in another. No cultures were made in the third case. Post-mortem blood cultures were negative in the case of overwhelming sepsis and lung cultures were not made. Microscopic findings, including pneumonia, supported this diagnosis. In one infant, hemorrhagic pneumonia was due to *Staph. aureus* and in one, to *Str. hemolyticus*. The infant with streptococcal septicemia also had hemorrhagic pneumonia.

Table IV shows the incidence of various types of infections associated with massive pulmonary hemorrhage in infants less than 1 year old. The incidence of pneumonia in patients with massive hemorrhage paralleled the incidence of pneumonia in infants dying in this period from all causes.

TABLE IV

Incidence of Infection with Massive Pulmonary Hemorrhage in 60 Infants Less Than 1 Year Old

	Cases	Total
Pneumonia		38
With infection elsewhere, including septicemia	20	
Without infection elsewhere	18	
No pneumonia		22
With infection elsewhere	8	
Without morphologic evidence of infection	14	
Blood and lung cultures negative	2	
Bacteriologic evidence of infection equivocal	12	
Blood or lung culture negative (only one culture taken)	5	
Blood culture positive, lung culture negative	3	
Cultures not taken	4	
Total		60

Pneumonia associated with infection elsewhere, or with septicemia, was present in 20 patients with massive pulmonary hemorrhage. The incidence of hemorrhage in this group varied according to age, occurring at 3 days to 2 weeks in premature infants and at 2 days to 10 weeks in full-term infants. As many cases occurred in full-term infants less than 7 days old as in those more than 7 days old. The most frequently cultured organisms were *Staph. aureus, E. coli,* and *Str. hemolyticus*. *Staph. aureus* and *E. coli* were cultured together in 2 cases.

Infection without pneumonia was present in one premature infant and in 7 full-term infants, all less than 10 days old. The organisms cultured were similar to those found in association with pneumonia. In 5 of the 8, no other factors were noted which might explain pulmonary hemorrhage, but in the other 3, kernicterus, severe intra-uterine

asphyxia with subdural hematoma, and tentorial tears with massive intracranial hemorrhage were present.

In 14 cases, no morphologic evidence of infection was present in the lungs or elsewhere. In 2 cases of kernicterus, heart and lung cultures were negative. In 12 cases, morphologic evidence of infection was lacking, but the bacteriologic data were equivocal (Table IV). These 12 included 2 cases of severe intra-uterine anoxia, 2 of intraventricular hemorrhage, 2 of excessive transfusion, 3 of "hemorrhagic disease of the newborn," one of kernicterus, and 2 of congenital heart disease. This group exemplifies factors which may be important in the pathogenesis of pulmonary hemorrhage without demonstrable infection.

Types of Hemorrhage and Associated Conditions. In most cases of massive hemorrhage, alveolar and interlobular septal hemorrhage occurred together, but in 15 instances only alveolar hemorrhage was seen, 3 in premature and 12 in full-term infants. Infection appeared to be the most important factor in all but 3 of the 15. These 3 were associated with kernicterus.

Among small hemorrhages, septal hemorrhage occurred alone in 12 (8 premature and 4 full-term) infants. All of the premature infants were less than 6 days old. Dyspnea was present in all 12, and in most it was a prominent symptom. Pulmonary edema was present also. In 3 infants the heart was dilated.

Sections from the lungs of 50 unselected stillborn infants were studied. Hemorrhage of some type was observed in 27 of these. In 4, some aeration was noted microscopically, and in none of these were hemorrhages extensive. Microscopically, septal hemorrhages were found in 20 infants; in 6 of these alveolar hemorrhage was not present. In 4 infants, extensive septal hemorrhages were present with no (or minimal) alveolar hemorrhage (Fig. 2). Alveolar hemorrhage was noted in 20, 5 of whom did not have septal hemorrhage. Severe alveolar hemorrhage with no (or minimal) septal hemorrhage was noted in 4 infants. The severe septal hemorrhage appeared to originate about large arteries and bronchi. In several instances, small areas of focal alveolar hemorrhage were seen, sharply delineated from surrounding atelectatic lung. Congestion of capillaries and veins was almost universal. In the majority of stillborn infants the cause of death was severe intra-uterine asphyxia. Amniotic sac contents could be seen in the lungs. There seemed to be no correlation between the degrees of aspiration of amniotic material and of congestion and hemorrhage. Of some interest were the small septal and pleural hemorrhages associated with islands of extramedullary hematopoiesis, some of which lay in

the outer layers of the venous wall. These foci were seen in cases of erythroblastosis fetalis, as determined by post-mortem criteria.

Structure of Intrapulmonary Veins in Newborn Infants

We have sought to evaluate the structural factors which may be concerned in the pathogenesis of pulmonary hemorrhage in newborn infants. Other writers have reported on the arteries. The available histologic technics reveal no structural differences between the capillaries of infants and those of adults. Therefore, our attention was directed toward the structure of the intrapulmonary veins, especially since hemorrhage about these veins is commonly seen in newborn infants. The development of the pulmonary veins has been discussed from various aspects by a number of workers,[11-17] but not with particular regard to the origin of pulmonary hemorrhage.

Veins studied were 150 to 300 μ in diameter. A comparison of veins of equal caliber in the various weight groups showed certain differences. These same differences may be found between large and small veins in any area of lung. The structure of the inner layer (intima plus media)* was noted, as well as the amount and distribution of its elastic tissue, its relative thickness, and the borderline between inner layer and adventitia. In regard to these points, it was possible to find real anatomical differences between the groups. No difference was observed within each group between stillborn infants and infants who had lived less than 2 days.

Structure of Intrapulmonary Veins in Stillborn Infants or Infants Less Than Two Days of Age (Table I)

In group 1, sections stained with hematoxylin and eosin and with aniline blue showed nothing but endothelium and an outer layer of loose collagenous tissue in the walls of the small veins. Elastic tissue stains revealed two or three thin, subendothelial, elastic tissue fibers (Fig. 3). In the arteries, the borderline between media and adventitia was clear and the elastic tissue in the media was dense.

In group 2, it was possible to distinguish in some veins a thin inner layer in the wall, just beneath the endothelium, with a diffuse borderline between this and the adventitia. Elastic tissue appeared more dense (more fibers and thicker fibers) than in group 1 (Fig. 4).

Group 3 revealed no great differences from groups 1 and 2. However, there was more elastic tissue in the walls of the veins (Fig. 5).

* The term inner layer is used to include intima plus media because the borderline between intima and media is not apparent in small intrapulmonary veins of newborn infants.

Group 4 showed a more compact inner layer in the wall of the vein. There were generally three or four elastic fibers in this layer and the borderline between the inner and outer layers was less diffuse than in the preceding groups (Fig. 6).

In group 5 the inner layer was thicker, more compact, and contained more elastic tissue than in group 4. The borderline between inner layer and adventitia was diffuse.

In group 6 the borderline between inner layer and adventitia was rather clear, and elastic tissue fibers appeared more dense than in group 5. The inner layer formed one-fourth to one-third of the thickness of the wall.

In group 7 the inner layer formed one-fourth to one-third of the thickness of the venous wall. The borderline between inner layer and adventitia was clear. More elastic tissue was present than in the preceding groups.

In the full-term infant there were small intrapulmonary veins in which the inner layer formed about one-third of the thickness of the venous wall (Fig. 7). The borderline between this and the adventitia was sharp. More elastic tissue was present in the venous wall in full-term than in premature infants but the amount of elastic tissue was still much less than that in the arteries.

The Rate of Development of Intrapulmonary Veins During the First Month of Extra-uterine Life

The inner layer of the venous walls in 1-month-old, full-term infants contains many closely packed elastic tissue fibers. The borderline between inner layer and adventitia is sharp and the outer layer of collagenous tissue is compact. These veins differ from arteries of comparable size (average outer diameter) in several respects. The elastic tissue layer is of almost equal thickness but the elastic tissue fibers are thinner and less undulating than in arteries. Internal elastic lamellae are not clear in the veins as they are in the arteries, and the walls of the veins are thinner. Collagenous tissue of the outer layer of veins is not as thick or dense as that in comparable arteries. However, the intrapulmonary veins of 1-month-old infants appear more like arteries than do the veins of 1-day-old infants. A great individual variation in the structure and development of the intrapulmonary veins occurs in the first month of extra-uterine life. This makes comparison between premature and full-term infants difficult. In the first 14 days of life some premature infants show development in structure and others do not. However, during the first month of life, development is faster in premature than in full-term infants. In 1-month-old premature in-

fants, intrapulmonary veins (in some cases at least) are anatomically like those of full-term infants; for example, the infants of group 1, 5 weeks old, showed intrapulmonary veins comparable to those in full-term infants of the same age.

Structure of Branching Points of Small Veins

About 500 slides from premature and full-term, newborn infants were reviewed to determine whether structurally weak points could be demonstrated in the intrapulmonary veins. The structure of the branching points of small veins and the relationship between lymphatics and small veins were of the most interest because it was here that hemorrhage sometimes was seen. In veins 100 to 200 μ in diameter, branches 50 to 100 μ in diameter often were seen emerging at right angles. When the diameter of the branch was about one-half that of the larger vein, its wall was about one-half as thick. In longitudinal sections elastic fibers continued unbroken from the vein to the branch. Therefore, it did not appear that weakness at the branching point was due to any discontinuity or thinning of elastic tissue.

Lymphatics commonly were associated with the branching points of the veins (Fig. 8). Miller[18] stated that lymphatics form a network around intrapulmonary veins. In his illustrations, lymphatics may be seen partially encircling the veins at some branching points. We paid particular attention to this feature. At the branching points of small veins associated with lymphatics, the distance between the endothelium of the lymphatic and that of the branched vein was variable. At some branching points, a thin elastic fiber intervened. In others the endothelia appeared to be adjacent; however, Foot's reticulum stain revealed one or two thin reticulum fibers intervening (Fig. 9). These formations were not universally present at branching points.

Lymphatics were seen adjacent to arteries, but there was always more tissue between the lumina of arteries and lymphatics than between those of veins and lymphatics (Fig. 10).

Discussion

Massive pulmonary hemorrhage in infants is uncommon, having occurred in only 67 of 4,000 cases in which necropsy was performed at the Children's Hospital Center between 1927 and 1946. It occurred more commonly in newborn infants than in older ones. It is not a distinct entity and may be associated with various conditions which themselves may cause death in the young. Small pulmonary hemorrhages are common in all infants.

Morphologic evidence alone is not sufficient to establish whether

massive pulmonary hemorrhage occurs terminally or some hours or days before death. Strassmann[19] stated that hemosiderin may appear 24 hours after aspiration of blood into the lungs of the rat, yet it was rarely seen in our cases. However, clinical signs consistent with pulmonary hemorrhage, such as appearance of blood in the nostrils, vomiting of blood, râles and dullness in the chest, were observed repeatedly several hours, and sometimes a day, before death. After signs of hemorrhage were noted the clinical course was progressively downhill. Therefore, it is believed that massive pulmonary hemorrhage may be a fatal complication of a variety of illnesses in early life.

Infection, kernicterus, transfusion, intracranial hemorrhage, congenital heart disease, and general debilitation (caused by malnutrition or occurring after operation) are factors which alone or, more commonly, in combination, may produce massive pulmonary hemorrhage.

The importance of infection associated with pulmonary hemorrhage in newborn infants has been pointed out by Ylppö[9] and Browne.[10] Cruickshank and Davidson[20] reported 5 cases of massive pulmonary hemorrhage in adults, associated with an infection of *Clostridium welchii* type. McCordock and Muckenfuss[21] produced hemorrhagic pneumonia with necrosis by intratracheal injection of varicella virus in rabbits. Our material suggests that massive pulmonary hemorrhage due to infection may occur in older as well as in newborn infants. Pneumonia accompanied massive pulmonary hemorrhage in 38 of 60 cases. Pneumonia with infection elsewhere also occurred frequently. The most commonly cultured organisms were *Staph. aureus, E. coli,* and *Str. hemolyticus*. These same three microorganisms were found by Dunham[22] to be associated most often with septicemia in her study of 59 cases in infants less than 1 month old. Browne found *Staph. aureus* and *E. coli* most commonly associated with massive pulmonary hemorrhage in 7 infants. Ylppö found *Staph. aureus* and *E. coli* most frequently, both in massive pulmonary hemorrhage and in septicemia, in newborn premature infants. Browne stated that massive pulmonary hemorrhage associated with infection was due to a sudden, overwhelming infection. Our material does not suggest this because, when infection has been an important associated finding, it has been present for several days before death.

Eight cases of massive pulmonary hemorrhage were found in which pneumonia was not present but in which there was infection elsewhere. In 5 of these no other factors which might account for the hemorrhage were present. This finding suggests, but does not prove, that pulmonary hemorrhage may be a manifestation of the initial stage of pulmonary inflammation.

In some instances, the site of the hemorrhage may be correlated with the clinical and pathologic data. Alveolar hemorrhage (without septal hemorrhage) occurs with infection and kernicterus. Capillary injury may be present in both, but the mechanisms producing it may differ. In the case of infection, direct injury by bacteria, viruses or their products may be of prime importance. With kernicterus, a neural mechanism may be in operation. Farber[23,24] has shown that pulmonary congestion and edema may be produced by vagal resection in the rabbit and guinea-pig. Kernicterus frequently does involve the nuclei of the medulla and damage to the central vagal nuclei may be equivalent to high vagotomy in man.[25] As will be seen later, however, many factors may work together to produce massive pulmonary hemorrhage in the newborn infant.

Subpleural and septal hemorrhages are seen associated directly with foci of extramedullary hematopoiesis in the vessel wall. The same phenomenon is seen in leukemia in which vascular infiltration has occurred. Of course, other bleeding factors may be present in both of these diseases.

The comparatively frequent occurrence of septal hemorrhage in the stillborn infant is interesting. Most of the septal hemorrhages in these infants appeared to arise from veins lying in the interlobular septa. In several instances the hemorrhage extended into the interlobular septa from periarterial and peribronchial tissue. It may have arisen directly from these arteries, from the vasa vasorum of the vessels derived from the bronchial arteries, or from the capillaries in the outer layer of the bronchial wall, derived from bronchial arteries.

Proft[26] suggested (but did not prove by experiments) that, in stasis of lungs, hemorrhages may occur from capillaries originating from bronchial arteries because the pressure in bronchial arteries is higher than that in the pulmonary circulation. This may be possible in certain large septal hemorrhages in stillborn infants when the hemorrhage extends from peribronchial tissue to the septa. It has been suggested also by Leff,[27] although not supported by experimental data, that in fetal asphyxia the fetus receives an increased amount of blood, due to contraction of the uterus and placenta. This may cause congestion and, finally, bleeding in fetal lungs. However, the circulation in the lungs during fetal life is not understood well enough to permit evaluation of this theory. The increased incidence of septal hemorrhage in stillborn and premature infants may be related to atelectasis.

Alveolar and septal hemorrhages are seen together more frequently in premature infants than in full-term and older infants. The intrapulmonary veins of premature infants are structurally weaker than

similar veins in full-term infants; those of newly born full-term infants are weaker than those of infants 1 month old. As age increases, the wall of the intrapulmonary vein becomes more like that of an artery. Perhaps congestion of the veins is more likely to produce perivenous and septal hemorrhages in premature infants than in full-term and older infants because of the relative weakness of the venous wall in the premature infant.

Venous congestion appears to be an important factor in the pathogenesis of pulmonary hemorrhage. Congestion of veins, capillaries, and, to a lesser degree, arteries, was seen in most of our cases of massive pulmonary hemorrhage. Also supporting this observation is the early occurrence of hemoptysis and occasional pulmonary hemorrhage in patients with mitral stenosis.[28,29] When Schlaepfer[30] produced partial occlusion of the pulmonary veins in the dog, severe pulmonary congestion and hemorrhage occurred, sometimes resulting in death within 2 days. Some correlation may be noted between the associated findings and pulmonary congestion in cases of massive pulmonary hemorrhage in infants. Congestion and edema of the lungs are seen almost universally in cases of intracranial hemorrhage in adults.[31] The occurrence of pulmonary hemorrhage with intracranial hemorrhage in full-term and premature infants has been mentioned previously in this paper. The relationship is not understood.

An increase in pulmonary venous and arterial pressure may occur with asphyxia, according to Johnson, Hamilton, Katz, and Weinstein.[32] Kountz and Hammouda,[33] by use of the heart-lung preparation in the dog, have shown that asphyxia leads to dilatation and later to slowing of the heart. Dilatation was observed first in the left side of the heart. Dilatation and slowing may be alleviated when asphyxia is relieved.

Operations in the chest have been associated with auricular flutter and fibrillation without detectable heart disease.[34,35] Abdominal operations may predispose a patient to atelectasis and pneumonia. Anesthesia, under some conditions, may produce asphyxia, according to Henderson.[36] These operative factors may be important in producing pulmonary congestion. Excessive transfusion may produce pulmonary congestion by overloading the circulation. Pulmonary hemorrhage, however, is not produced in dogs by excessive transfusion.[37] In cats with partial pneumonectomy, transfusion produces pulmonary edema.[38]

Asphyxia has been mentioned previously in relation to pulmonary congestion. It has been shown that capillary permeability is increased with asphyxia.[39,40] Many factors, such as lack of oxygen and increased acidity,[7,8,40-42] may help to explain this mechanism. Asphyxia, or factors which can produce it, were frequent findings in our cases of mas-

sive pulmonary hemorrhage, but asphyxia is common in this age group in any event. It has not been shown that increased capillary permeability has any direct influence on capillary hemorrhage.

Except for direct injury to capillaries, pulmonary congestion and the factors which produce it seem most important in the pathogenesis of pulmonary hemorrhage in the newborn infant. The higher incidence of pulmonary hemorrhage in the newborn than in the older infant may be related to the comparative weakness of the left side of the heart in the neonatal period.

Multiple pulmonary emboli and thrombi, resulting postoperatively in severe pulmonary hemorrhage, as described by Cutler,[43] were not encountered in our study. Recent thrombi were seen occasionally in small vessels. The rôle of thrombosis of pulmonary vessels in producing hemorrhage cannot be evaluated properly in this study.

The major change in structure of the intrapulmonary veins in infants during growth from 450 to 1,500 gm. is in the amount of elastic tissue present in the inner layer of the venous wall. In infants weighing from 1,500 to 2,500 gm., the elastic tissue of the veins increases similarly, but the greatest change lies in the sharpness of the borderline between inner and outer layers. Aniline blue stains show this change best.

Summary

Massive pulmonary hemorrhage was found in 67 of 4,000 infants seen at post-mortem examination. It occurred more frequently in newborn infants, with 77.5 per cent of the cases occurring in infants less than 15 days old. It may occur adjacent to nearly every part of the intrapulmonary vascular system; arteries, capillaries, veins, and bronchial arteries. In practically all cases, massive pulmonary hemorrhage arises from alveolar capillaries and from veins in the interlobular septa. Massive alveolar hemorrhage, without septal hemorrhage, is seen more commonly in infants living at birth than in stillborn infants. On the other hand, large septal hemorrhage without alveolar hemorrhage is found in stillborn infants more frequently than in infants living at birth. Septal hemorrhages usually originate from the septal veins but are seen occasionally about pulmonary arteries and may originate from the vasa vasorum of those vessels which are part of the bronchial circulation. Capillary rupture sometimes is demonstrable in cases of alveolar hemorrhage. In cases of massive pulmonary hemorrhage, alveolar hemorrhage (without septal hemorrhage) is seen more often in the full-term than in the premature infant.

Massive pulmonary hemorrhage is not a distinct entity, but may occur as a fatal complication of several severe diseases in infancy, the

most common being infection, intracranial hemorrhage, kernicterus, and congenital heart disease. In most cases it is not an agonal phenomenon. Infection is more frequently associated than any other condition with massive pulmonary hemorrhage in infants. Organisms most commonly cultured include *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus hemolyticus*. Pulmonary congestion appears to be an important factor in the pathogenesis of massive pulmonary hemorrhage.

The structural differences between intrapulmonary veins and arteries become less distinct with increasing age of the fetus and continue to disappear after birth. In premature infants weighing less than 750 gm. at birth, the small intrapulmonary veins consist of endothelium with one or two thin subendothelial elastic tissue fibers surrounded by a layer of loose collagenous tissue. During fetal life the inner layer of the venous wall becomes thicker and more compact, and elastic tissue fibers of the inner layer are increased in number and thickness. In the full-term infant there is a sharp borderline between inner layer and adventitia, and the inner layer forms about one-third of the thickness of the venous wall. After birth, elastic tissue and other elements of the inner layer grow continuously, especially in premature infants during the first month of life. The lymphatics of the vein at the branching points of the vessel are separated from the lumen of the vessel in many instances by only a small amount of elastic tissue or reticulum fibers. Anatomically, this appears to be a weak point in the venous system.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Massive periarterial hemorrhage in two layers, observed after large transfusions on 2 successive days.

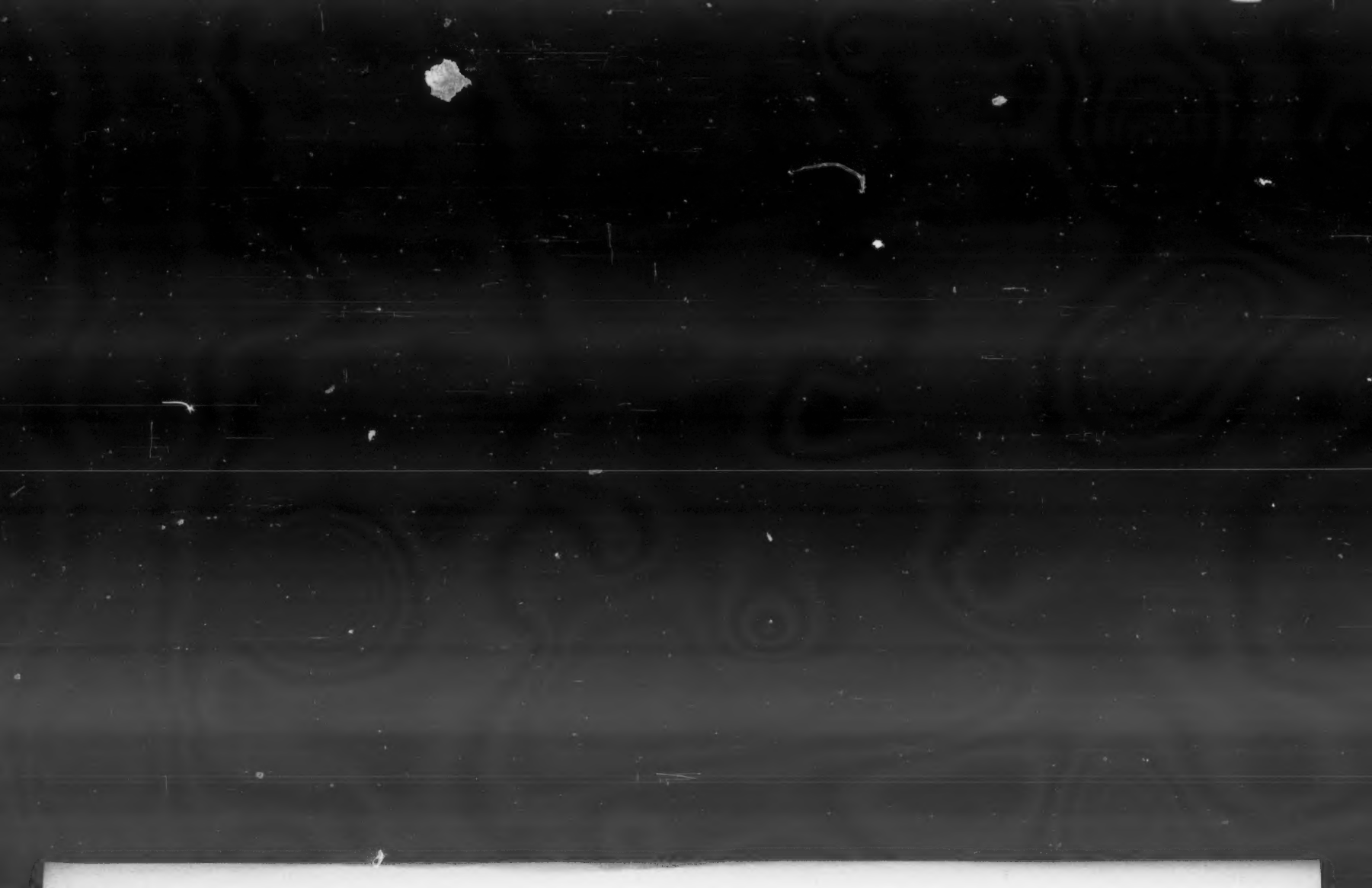
FIG. 2. Massive septal hemorrhage in a premature infant 7 days old, weighing 1,800 gm. (4 lbs.) at birth. Hemorrhage is absent in alveoli and lymphatics. The lungs are incompletely aerated. Alveoli contain alveolar phagocytes and precipitated material.

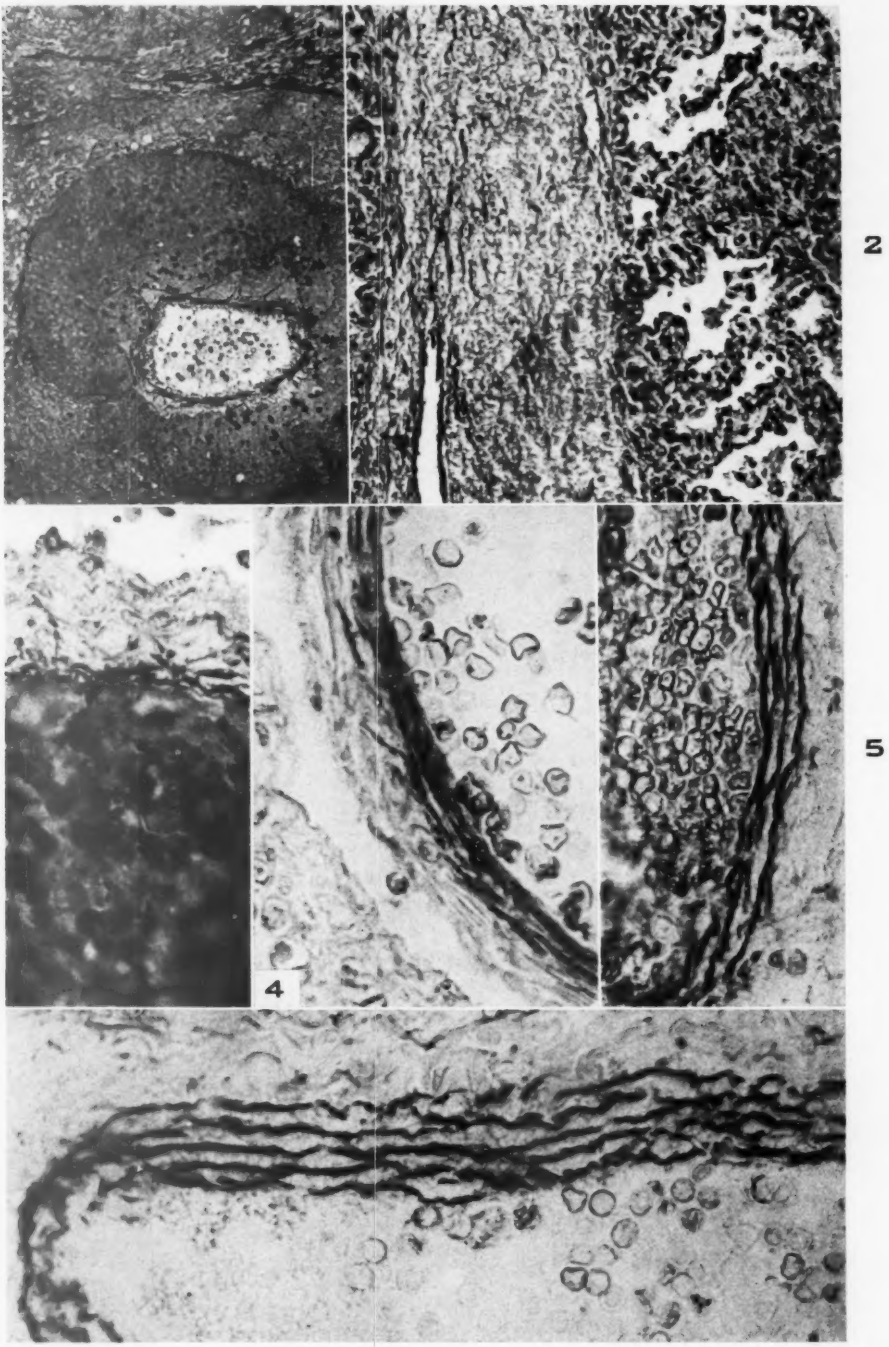
FIG. 3. Intrapulmonary vein from infant 1 day old with birth weight of 590 gm. (1 lb., 5 oz.). The subendothelial layer of elastic tissue is scanty and the wall of the vein flimsy. Elastic tissue stain.

FIG. 4. Intrapulmonary vein from infant 1 day old with birth weight of 1,180 gm. (2 lbs., 10 oz.). Three to four layers of elastic tissue are present in the inner layer of the venous wall. Elastic tissue stain.

FIG. 5. Intrapulmonary vein from infant 2 weeks old with birth weight of 1,120 gm. (2 lbs., 8 oz.). The elastic tissue is developed almost as extensively as it is in the newborn full-term infant (see Fig. 6). Elastic tissue stain.

FIG. 6. Intrapulmonary vein from full-term infant 1 day old. The elastic tissue consists of many dense fibers in the inner layer of the venous wall. Elastic tissue stain.





Ahvenainen and Call

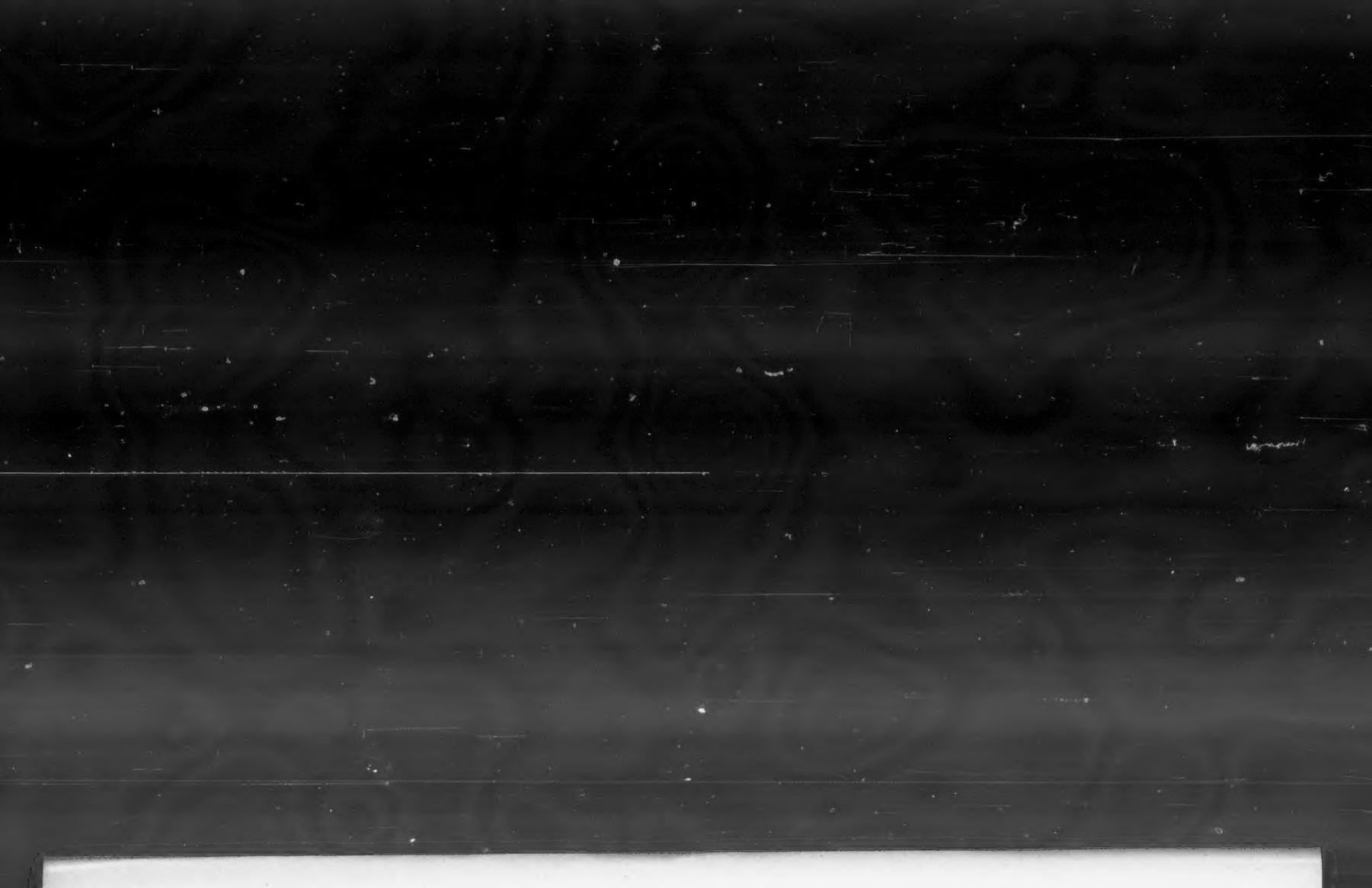
Pulmonary Hemorrhage in Infants

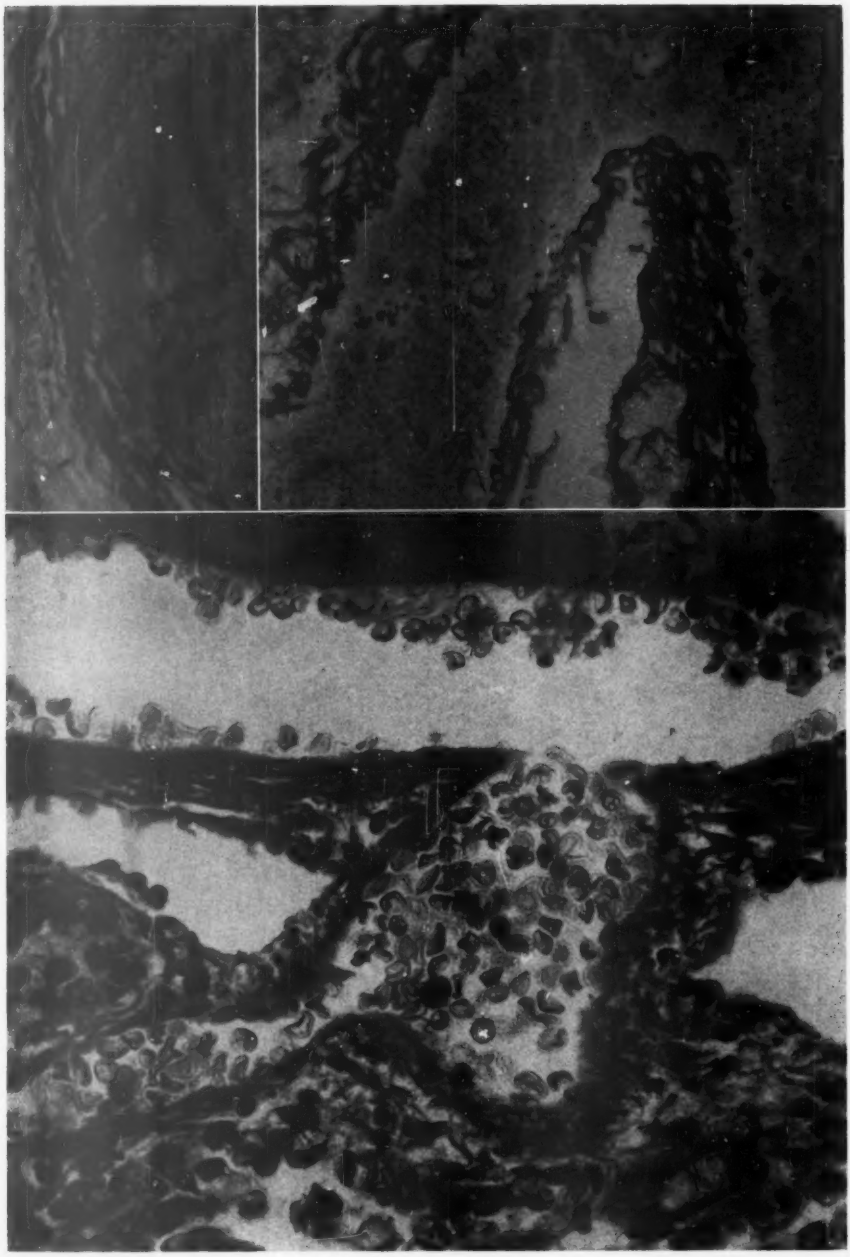
PLATE 2

FIG. 7. Intrapulmonary vein from infant 1½ days old, with birth weight of 2,140 gm. (4 lbs., 12 oz.). The layers of elastic tissue are fairly dense and compact in the inner portion of the venous wall. Elastic tissue stain.

FIG. 8. A septal vein at the branching point, showing adjacent lymphatic. Verhoeff's elastic tissue stain.

FIG. 9. A septal vein at the branching point, showing adjacent lymphatic and a flimsy layer of reticulum tissue between the lumen of the vein and that of the lymphatic. Foot's reticulum stain.

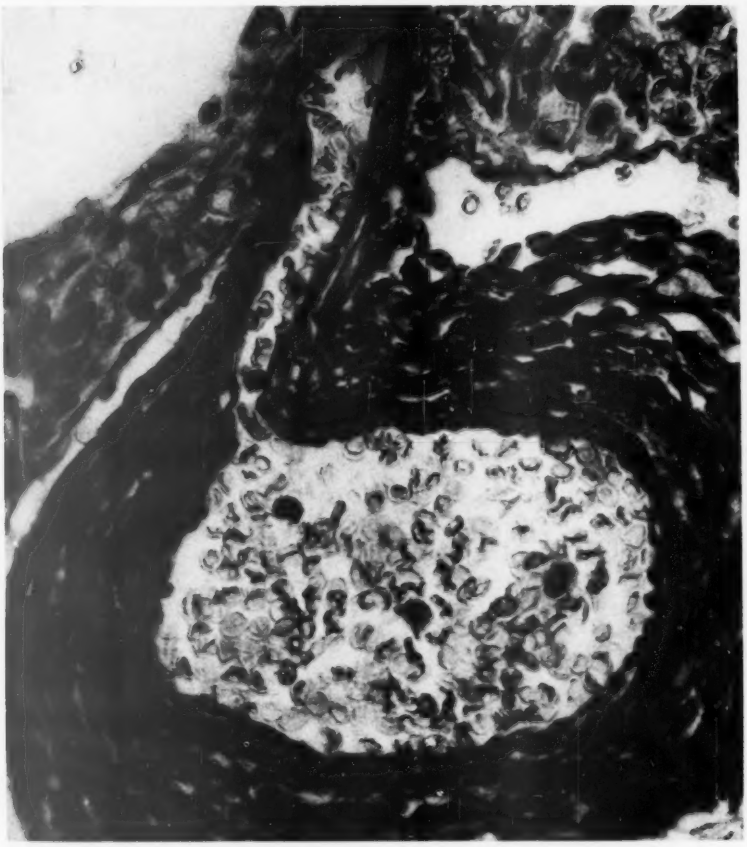




Ahvenainen and Call **Pulmonary Hemorrhage in Infants**

PLATE 3

FIG. 10. Intrapulmonary artery at the branching point showing relationship to surrounding lymphatic. At this point no area of weakness is noted like that seen in the vein.



10

THE EFFECT OF AUREOMYCIN ON THE RADIATION SYNDROME IN DOGS*

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Following a large dose of whole body x-radiation, a disease syndrome occurs which has certain characteristics common to most species. The signs and symptoms of the radiation syndrome have been amply reviewed by many authors.[1-4] The most pronounced derangement is a hemorrhagic diathesis which occurs to a major or minor extent and results in a diminished blood volume. Severe damage to major organs may result from extravasation of blood into or around these organs. Accompanying this bleeding tendency is a pan-hematopenia secondary to the destruction of the formed elements of the blood and the relative aplasia of the bone marrow. Associated with damage to the reticulo-endothelial system, humoral antibody response to bacterial antigens is markedly diminished following certain doses of x-radiation.[5] These factors suggest that the integrity of the barriers to bacterial infection is lost after large doses of x-radiation and that bacterial infection is an important element in the morbidity and mortality of the acute radiation syndrome.

Warren and Whipple[6] demonstrated positive blood cultures in dogs that had received lethal x-radiation to the abdomen, but commented that the expected overwhelming tissue invasion by intestinal bacteria was not found. They pointed out that there may be a more important protective mechanism than the intact intestinal epithelium. Sepsis in mice following whole body x-radiation or neutron radiation has been demonstrated by Chrom[7] and Lawrence and Tennant.[8] Chrom attributed this to damage of the reticulo-endothelial barriers since sepsis did not occur when the liver and spleen were shielded during radiation. Studies made previously in our laboratory indicated that 17 per cent of 54 dogs that died after receiving 350 to 450 r. of whole body x-radiation had a positive blood culture 24 hours before death.[9] Miller, Hammond, and Tompkins[10] have reported results of blood and splenic cultures from mice that received whole body x-radiation. They suggested that bacterial infection of intestinal origin is an important factor in mortality.

Burrows, Deupree, and Moore[11] have shown that when normal

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guinea-pigs are infected with the cholera vibrio, the infection remains limited to the intestinal tract. However, following large doses of whole body x-radiation, the infection spreads to various other tissues. As a partial explanation of this, Burrows, Deupree, and Moore[12] have shown that there are alterations in serum and fecal antibody titers in guinea-pigs following radiation.

An x-radiated animal is further hampered in controlling infection by a severe leukopenia. However, the leukopenia is observed for several days before the clinical signs of infection become evident. It is of interest to note that dogs which survive a large dose of whole body x-radiation have shown as severe a leukopenia as those which died.[13]

A previous report[14] from this laboratory indicated that antibiotics are of value in alleviating the diarrheal state in rats and in reducing morbidity and mortality in dogs that have received large doses of x-radiation. Miller, Hammond, and Tompkins[15] reported that the use of streptomycin and other antibiotics reduces the mortality in x-radiated mice. This they attributed to the effectiveness of the antibiotics in controlling bacteremia. McDonnel and Maxwell[16] treated the radiation syndrome in dogs with aureomycin and found that it apparently reduces mortality.

This report concerns observations made on a group of 24 dogs that received a large dose of whole body x-radiation and were treated with a single antibiotic, aureomycin. Concurrent hematologic, bacteriologic, and pathologic studies were made, including an evaluation of the clotting mechanism.

Dogs treated with aureomycin alone, with no other supportive therapy such as blood transfusions, have shown delay in the onset of morbidity and mortality following x-radiation as compared with control dogs. In this study there has been a slight reduction in mortality in the treated dogs. It is of interest to note that total mortality varies from one study to another in this laboratory, whereas delay in onset of morbidity and mortality has been observed consistently in every study. Pathologic studies revealed no ulceration and only minimal bleeding tendencies in the intestinal tracts of those treated dogs that died, while the control animals that died showed massive gastro-intestinal hemorrhage and extensive ulceration of the small intestine.

Methods

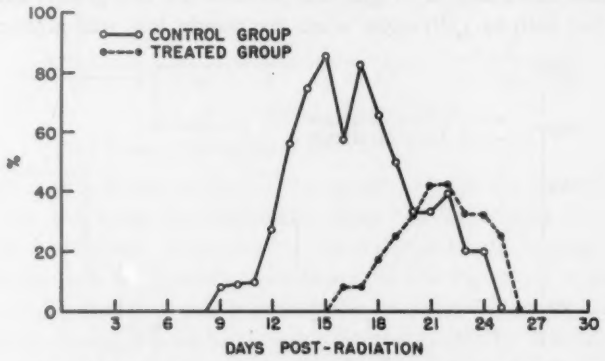
The dogs used were adult healthy mongrels averaging 11.3 kg. They were housed in individual cages and fed a soft mash diet.* As the ani-

* Purina dog chow, kibbled meal.

mals became anorexic for the mash following radiation they were offered fresh ground beef and milk. Twenty-four dogs were divided in two groups of 12 each. Dogs of the treated group were paired with dogs of the control group according to weight and build, so that the two groups were comparable.

Radiation was administered with a 250 kv. Picker x-ray machine at 15 ma., using a parabolic aluminum filter with 0.5 mm. of copper. The target skin distance was 40 inches and the total target skin dose was 450 r., administered at the rate of 7.15 r. per minute. The dogs were exposed in pairs composed of one dog from each group. Immediately following radiation, each of the 12 dogs in the treated group received aureomycin* and this medication was continued every 6 hours, day and night, for 28 days (approximately 100 mg. of aureomycin per kg. per 24 hours). The aureomycin capsule was given in a small ball of hamburg; each control dog received a similar ball without aureomycin.

Bacteriologic studies included cultures of blood, feces, and tissues obtained by necropsy. Saline suspensions of feces were used in making pour plates so that quantitative counts of the bacteria could be made. Fecal bacteria were isolated on selective media for identification of groups of bacteria and no attempt was made to differentiate



Text-figure 1. Percentage of dogs with diminished activity.

species. The sensitivity to aureomycin of the bacteria isolated was determined. Hematologic studies included counts of the red blood cells, white blood cells, and platelets. Clinical observations included appetite, weight, activity, and bleeding tendencies. Any dog that showed definite lethargy was recorded as having diminished activity and this datum was used in constructing Text-figure 1.

*Supplied by Lederle Laboratories, American Cyanamid Company, Pearl River, New York, through Dr. Stanton M. Hardy.

A complete necropsy, excluding the cranial cavity, was performed on each dog that died. Color photographs were taken of typical and unusual gross pathologic changes.

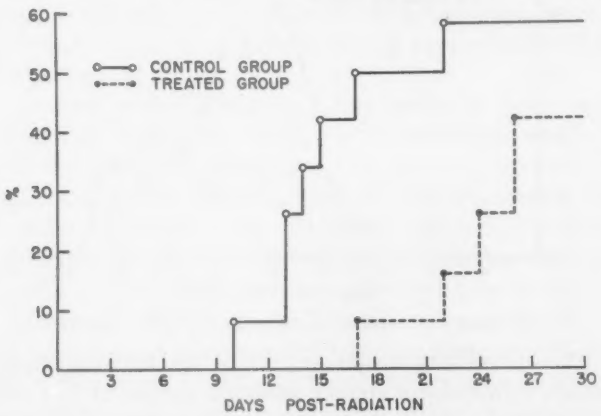
All studies began 14 days before x-radiation and continued for 28 days following irradiation.

Results

Although weakness, lethargy, and anorexia, symptoms typical of radiation sickness in the dog, were as severe in the treated as in the control dogs, they were evident over a shorter period of time. Text-figure 1 shows that lethargy was first noted in the control dogs by the 9th or 10th day post-radiation and by the 10th to 16th day none of these dogs was eating and all appeared very lethargic. In marked contrast, the treated group appeared outwardly normal during this period; anorexia and lethargy did not appear until the 17th day post-radiation and continued to the 25th day in the surviving dogs.

There was no essential difference in the extent of subcutaneous hemorrhagic areas between the two groups. No diarrhea was noted in either group except when gross bleeding from the bowel occurred.

Following radiation, all dogs began to lose weight and there was no significant difference in weight loss between the two groups except between the 10th to 15th days, when the weight loss was greater in the



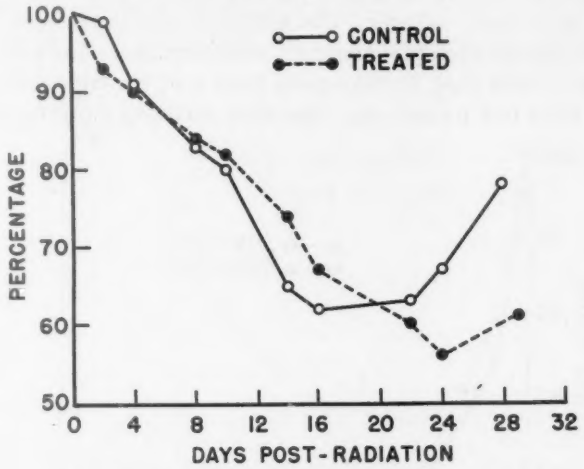
Text-figure 2. Cumulative mortality by days expressed as percentage of number in each group.

control group. It was during this period that the greatest incidence of mortality occurred in the control group. The average weight loss in both groups was approximately 15 per cent of pre-radiation weight.

One of the more striking observations was that all deaths occurred

significantly earlier in the control group than in the treated group. Text-figure 2 shows that the first control dog died on the 10th day post-radiation, whereas the first death in the treated group did not occur until the 17th day post-radiation. In this series the total mortality at 4 months post-radiation was 58 per cent for the control group and 44 per cent for the treated group.

Hematologic studies showed the typical rapid drop in white blood cell count and the somewhat delayed drops in red blood cell and platelet counts. These are shown in Text-figures 3, 4, and 5. The red blood



Text-figure 3. Percentage of pre-radiation red cell count.

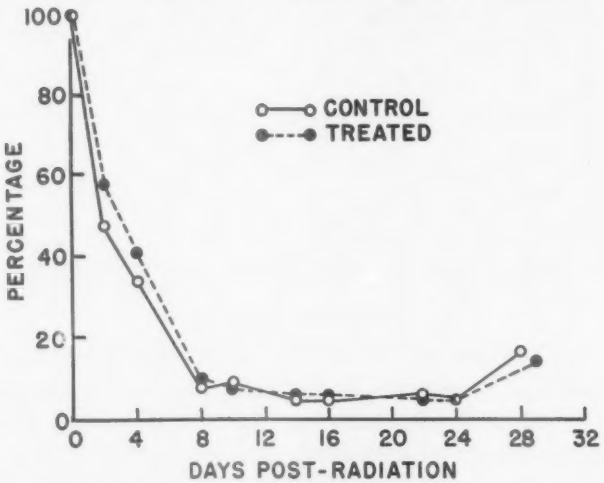
cell counts were lower in the control group than in the treated group during the 3rd week post-radiation. This can be related directly to the higher incidence of gastro-intestinal hemorrhage in the control dogs. There was no significant difference in the degree of leukopenia. In all animals that died, the white blood cell count fell to 300 cells per mm. or less during the 24 to 48 hours preceding death. No significant difference was observed between the blood platelet counts. The first evidence of bleeding tendency occurred on the 12th to 14th days at which time the platelet counts were observed to be below 30 per cent of the pre-radiation value. As shown in Text-figure 6, the coagulation times increased in both groups following radiation but there was no significant difference between the groups, and no significant variation occurred immediately preceding death in the dogs that died. Coagulation data were obtained on only 10 of the dogs studied.

Details of the bacteriologic studies of the blood, feces, and necropsy material will be presented in a forthcoming issue.[17] The percentage of

positive blood cultures in the control group increased following radiation and exceeded the number of positive cultures in the treated group, as shown in the following table:

	Pre-radiation (aerobes and anaerobes)	Post-radiation (aerobes and anaerobes)
Control	1.4% positive	14.8% positive
Treated	8.5% positive	6.3% positive

More facultative and obligative anaerobes than strict aerobes were isolated from these cultures. The following organisms were isolated from cultures of necropsy material: colon-aerogenes from all of the control dogs that died, Pseudomonas from 2 of the treated dogs, and Proteus from one treated dog. Negative necropsy cultures were ob-

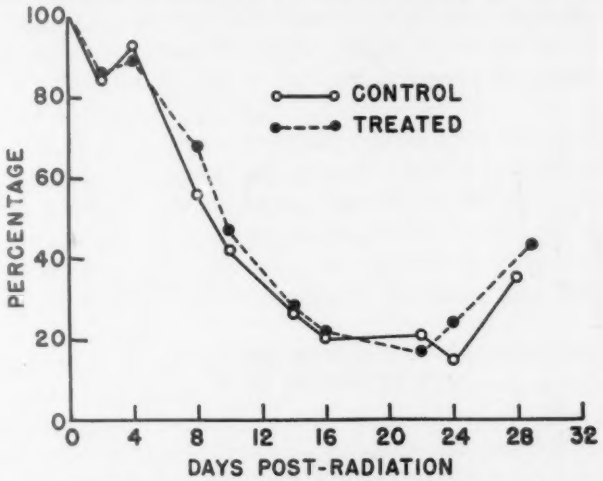


Text-figure 4. Percentage of pre-radiation white cell count.

tained from 2 of the treated dogs that died. Sensitivity tests revealed that the organisms isolated from necropsy material from the control dogs were sensitive to aureomycin, whereas those isolated from the treated group were resistant.

Quantitative analyses of the bacterial flora of the feces showed that the coliform organisms increased markedly in both groups following radiation. It is interesting that the coliforms reached significantly higher levels in the treated than in the control group. Less marked increases were observed in the counts of staphylococci and streptococci in both groups. The incidence of Proteus in the treated groups increased post-radiation. No pathogenic organisms were isolated.

The most prominent gross finding at necropsy was evidence of a hemorrhagic diathesis with extravasation of blood in the lungs, spleen, lymph nodes, kidneys, and mucosa of the gastro-intestinal tract, and beneath the pleura and peritoneum. Bleeding in the control group was more extensive and varied, occurring in more organs than in the treated groups. Six of the 7 control dogs that died had large amounts of blood in the gastro-intestinal tract at necropsy, associated with ulceration of the mucosa of the stomach and small intestine. The 5 treated dogs that died showed no ulceration of the intestinal mucosa and no blood within the lumen of the intestine. The differences in degree and extent of hemorrhage for the two groups may be related to



Text-figure 5. Percentage of pre-radiation platelet counts.

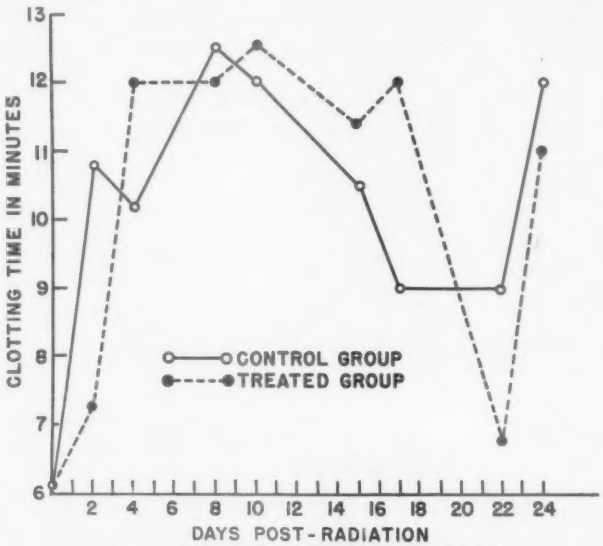
the earlier death in the control dogs. The treated dogs died at a later period when the bleeding tendency was abating, as evidenced by observations of the clotting times and platelet counts.

Discussion

Following a large dose of whole body x-radiation, certain characteristic abnormal signs appear which are seen in most species of animals. These signs reflect the essential physiologic derangements resulting from the effect of x-radiation on various tissues. In studies on any general toxic agent, individual differences in degree of response are noted among animals which have been exposed to equal doses. This is found to be true of animals exposed to a large dose of x-radiation and is probably due to indeterminable inherent variation within the animal. In particular, differences in degree of lethargy and anorexia are found,

but the fundamental pathologic changes in body function tend to be identical. One of the most uniform is a profound depression of the hemopoietic tissue in the bone marrow, which in turn causes a diminution in the peripheral blood elements. Therefore peripheral blood counts can be used as an index of the severity and uniformity of the dose of x-radiation. All dogs observed in this study showed almost identical depression of erythrocytes, leukocytes, and platelets.

Although all dogs received a uniform dose of x-radiation, and although individual variation in response may occur, a consistent and significant difference in the time of onset and severity of the signs of radiation toxicity was observed between the control and treated groups.



Text-figure 6. Average clotting time in minutes.

Since aureomycin was the only specific therapeutic agent administered in this study, presumably the delayed morbidity and mortality of the radiation syndrome in dogs can be attributed to this antibiotic. Since aureomycin is a potent antibiotic with a broad anti-bacterial and anti-viral spectrum, bacterial and possibly viral infection may be associated with the toxicity produced by a large dose of x-radiation. Such a concept is supported in part by the bacteriologic studies made in this experiment.[17] From cultures of necropsy material from the control dogs, aureomycin-sensitive organisms were isolated, whereas from treated dogs only aureomycin-resistant bacteria were isolated.

The maintenance of normal activity in the aureomycin-treated dogs

may not be beneficial to these animals since there is evidence that excessive activity in x-radiated animals increases the mortality. Kimeldorf, Jones, and Fishler[18] have shown that physical stress increases the mortality in x-radiated rats. During the period of pronounced bleeding tendency, it is conceivable that activity resulting in only minimal trauma to exposed parts of the body may be responsible for initiating or extending the subcutaneous purpura, and may be partly responsible for internal bleeding.

Also, physical activity may increase catabolism excessively in x-radiated animals, which is undesirable. Some of the treated dogs began to lose weight before anorexia was evident. This may be related to the continuation of their normal physical activity. The lethargy exhibited by the x-radiated animals may be secondary to the primary effects of x-radiation such as toxemia and anemia. Restriction of the normal physical activity displayed by the treated dogs is being contemplated for future studies.

Ulceration of the gastro-intestinal mucosa with massive gastro-intestinal hemorrhage in 6 of the 7 control dogs that died, and the absence of ulceration and minimal gastro-intestinal hemorrhage in the treated dogs that died, suggest that the changes in bacterial flora of the bowel, occurring during the administration of aureomycin, tended to prevent these lesions of the intestinal mucosa usually found in the radiation syndrome.

It has been reported that aureomycin shortens whole blood coagulation time.[19] This effect could conceivably benefit the radiated animal by decreasing the hemorrhagic diathesis. However, studies in this laboratory show that aureomycin administered orally in therapeutic dosage has no effect on the whole blood coagulation time, while intravenous administration of aureomycin may produce a remarkably prolonged coagulation time.[20]

Aureomycin may play a more complex rôle in the radiation syndrome than that of combatting infection. Studies in this laboratory indicate that aureomycin-treated rats that survive x-radiation regain weight more rapidly than do the controls. Jukes *et al.*[21] have reported a growth-promoting effect of aureomycin on pigs. It is possible that some of the signs of radiation sickness are evidence of abnormal detoxification processes, and that the reduced morbidity in the treated dogs may be due to an antitoxic effect of aureomycin. This antibiotic not only may have a bactericidal effect, but may also play a rôle in destroying bacterial toxins.

Further work is needed to determine the optimal dose of aureomycin

and the post-radiation period when the antibiotic will be most effective. McDonnel and Maxwell[16] have reported better results using a lower dose of aureomycin, *i.e.*, 250 mg., twice daily; their animals, however, received much less handling following radiation. Preliminary studies in this laboratory, in which the antibiotic therapy was withheld until clinical signs of radiation sickness had developed, have shown that delayed therapy is not of benefit to the radiated dog. The possibility of a toxic effect of this drug after extended use has not been investigated. Larger amounts of the drug may be absorbed from the damaged intestinal mucosa with its increased permeability.

Fundamentally contributing to individual mortality in the radiation syndrome are: infection, derangements of the blood-clotting mechanisms, pan-hematopenia, and metabolic disturbances such as increased catabolism and negative electrolyte balance. Aureomycin is effective in preventing and combatting infection. Other antibiotics may prove more potent.[22] Blood transfusions, anticoagulants, fluid, and electrolyte therapy are examples of other therapeutic approaches necessary for more complete alleviation of radiation sickness.

The delayed onset of morbidity and mortality in the aureomycin-treated animals that had received a large dose of x-radiation should be useful in providing added time for the institution of other therapeutic measures.

Summary

Twelve dogs that had received 450 r. of whole body x-radiation were treated with aureomycin following radiation. Twelve similarly x-radiated dogs served as controls.

Identical alterations in blood cell counts and coagulation mechanisms were observed in both groups.

Bacteriologic studies showed a higher percentage of positive blood cultures in the control dogs than in the treated dogs.

Bacteriologic studies of the feces showed a shift in bowel flora with an increase in the number of coliform bacilli and staphylococci. The increase was most marked in the treated dogs.

Organisms recovered from cultures of necropsy material from the control dogs were sensitive to aureomycin while those from the treated dogs were resistant.

Gross necropsy findings revealed no gastro-intestinal ulceration and minimal evidence of hemorrhage in the treated group while marked gastro-intestinal ulcerations and hemorrhage were found in the control dogs.

The total mortality was 58 per cent in the control group and 44 per

cent in the treated group. The first death in the control group occurred 7 days before the first death in the treated group. A similar delay in the onset of the clinical signs of radiation sickness was noted.

It is postulated that the observed changes are related to the control of the infection and infectious processes by aureomycin.

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EFFECTS ON THE WHITE MOUSE OF A SINGLE WHOLE-BODY EXPOSURE TO 190 MEV. DEUTERONS*

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With the availability of 190 mev. deuterons from the 184 inch cyclotron, several investigators in this laboratory became interested in the possibility of delivering radiation to selected organs or tumors in animals and humans using the properties of the Bragg ionization curve.[1,2] Qualitatively, one expects to find the same type of histologic effects with high energy protons or deuterons as with x-rays. Moderately high energy fast neutrons, acting chiefly through proton recoils, produce histologic changes similar to those due to x-rays, as the studies of Lawrence and Tennant[3] first demonstrated. It still seemed worth while to initiate a preliminary study of the histologic effects caused by high energy deuterons in order to proceed to the quantitative aspects of the problem, that is, the effectiveness of different portions of the Bragg curve. The studies here described were pursued in June, 1948, and they form a part of more extensive work on whole body irradiation of white mice and of mouse tumors. Detailed physical and biologic considerations on the use of high energy deuterons and alpha particles are presented in another communication.[4] The data presented here are confined to a limited investigation of tissue and blood changes occurring in the white mouse after a single whole body exposure to this deuteron beam. For comparison of these observations with others reported after exposure to x-rays and neutrons, there are several original papers and reviews available.[3,5-7]

MATERIAL AND METHODS

White mice of the Strong A strain were employed. They were housed and maintained under uniform standard conditions. From a pool of approximately 100 such mice, 20 were selected at random for preliminary blood studies which were completed during the 72 hours prior to exposure of the experimental group to the deuteron beam. These blood studies included enumeration of erythrocytes, leukocytes, and platelets, and differential counts on smears stained by Wright's method. The calculated mean values were employed to establish the normal base lines shown in Text-figures 1 to 6.

On June 13, 1948, three groups of mice were irradiated. Group I,

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† On leave of absence from the New Britain (Conn.) General Hospital.

consisting of 13 mice, received 3.8×10^4 ergs/gm.* Group II, consisting of 12 mice, received 7.5×10^4 ergs/gm. Group III, consisting of 15 mice, received 14.9×10^4 ergs/gm. Each group contained a similar number of controls.

The body weights of all treated mice were recorded at frequent intervals for comparison with pre-treatment values. Systematic examinations of the blood were carried out on the 1st, 3rd, 5th, 10th, and 18th days after treatment. At these time intervals 2 mice were selected at random from the survivors of each of the three groups, and 2 untreated mice were similarly selected from the control pool. Complete blood studies were conducted on these animals, after which they were sacrificed for tissue examination. Since the experimental groups were continually depleted by sacrifice and spontaneous death, observations to the 18th day were possible only on group I. Group II had no survivors beyond the 10th day, and group 3 none beyond the 4th day.

Mice that died spontaneously during the course of the experiment were studied histologically. Other mice, obviously moribund, were sacrificed terminally for similar studies. Brain, spinal cord, vertebra, sternum, liver, spleen, lymph nodes, skin, thymus, intestine, stomach, gonads, heart, and lung were fixed in 10 per cent formalin or in Bouin's solution and stained with hematoxylin and eosin. It was possible to prepare satisfactory bone marrow sections from the sternum without preliminary decalcification. Continual reference to control tissues was made throughout the histologic studies.

In Text-figures 1 to 6, the abscissas denote time on a logarithmic scale. Each point in the curves of blood findings represents an average of two observations, indicated as a percentage deviation from the pre-treatment normal control value. In group I there was one survivor at 23 days. The observations on this animal were combined with the 18 day value.

Results

Survival Time

Table I shows duration of life after bombardment. Estimation of survival time was complicated slightly by the fact that animals were killed from each group for tissue studies, but in spite of this it is evident that survival was related closely to total dosage. Of 5 animals in Group I surviving the 5th day, none died spontaneously thereafter, in contrast to 4 spontaneous deaths among the 5 animals in group II that were alive on the 5th day after treatment. Three mice from group

* 1 roentgen equivalent physical = 93 ergs/gm.

TABLE I

Number of Mice Surviving a Stated Period Following Bombardment

Days after bombardment	Group I		Group II		Group III	
	Dead	Surviving	Dead	Surviving	Dead	Surviving
1	2 (S)	11	2 (S)	10	2 (S)	13
2	0	11	0	10	0	13
3	2 (S)	9	2 (S)	8	2 (S)	11
4	0	9	1 (D)	7	11 (D)	0
5	2 (S), 2 (D)	5	2 (S)	5		
6	0	5	0	5		
7	0	5	0	5		
8	0	5	2 (D)	3		
9	0	5	2 (D)	1		
10	2 (S)	3	1 (S)	0		
11	0	3				
18	2 (S)	1				
19	0	1				
23	1 (S)	0				

S = Sacrificed for blood and tissue studies.
D = Died or moribund and sacrificed.

I survived the 10th day, while all animals in group II were dead at this time. Group III, receiving the highest dose, had no survivors after the 4th day.

Body Weight

Text-figure 1 depicts the change in body weight following bombardment. The average pre-treatment weight of the control group was 28.1 gm.; of group I, 27.7 gm.; of group II, 30.2 gm.; and of group III, 30.7 gm. There are no significant differences among these values. All groups showed weight loss following exposure to the deuteron beam in direct relation to dosage. Group I which received the smallest exposure had the smallest initial average weight loss with a return to normal on the 18th day. Group III, exposed to the heaviest dose, had the greatest average weight loss at 4 days when its last surviving member died.

Gross Findings at Necropsy

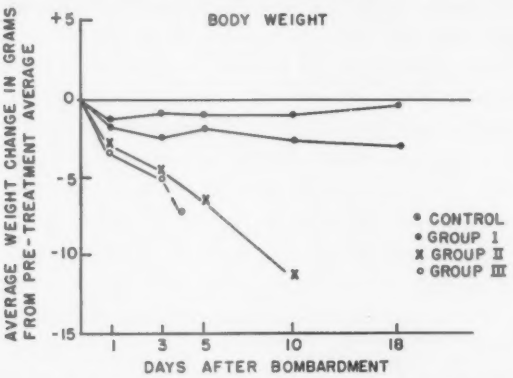
Animals reacted to the toxic effects of the beam by losing weight, developing ruffled fur, becoming listless, and finally exhibiting diarrhea. At necropsy after spontaneous death, there was generalized pallor of tissues, particularly of the liver, and watery yellow material in the stomach and intestinal tract. The spleen was markedly atrophic, and lymph nodes and thymus were so strikingly reduced in size that some-

times they were difficult to locate. Animals in group I surviving the 10th day showed no significant gross tissue changes.

Hematologic Alterations

The blood findings are summarized in Text-figures 2 to 6.

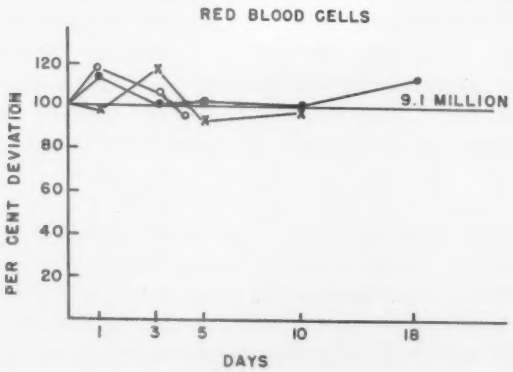
Red Blood Cells. Employing the normal pre-treatment average as a base line, an early slight increase was noted in all groups. Following



Text-figure 1. In Text-figures 1 to 6, time in days after bombardment is indicated on the horizontal axis on a logarithmic scale. For each attribute the observed values are plotted as a percentage of the normal value as determined from the control group.

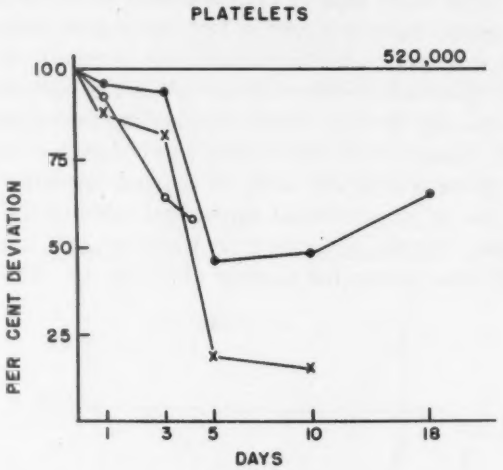
this initial rise, there was no significant deviation in the average red cell count of any of the three treatment groups during the observation period of 2½ weeks (Text-fig. 2).

Platelets. A precipitous drop in the platelet count was observed in all three groups, as can be seen in Text-figure 3. This was most marked



Text-figure 2.

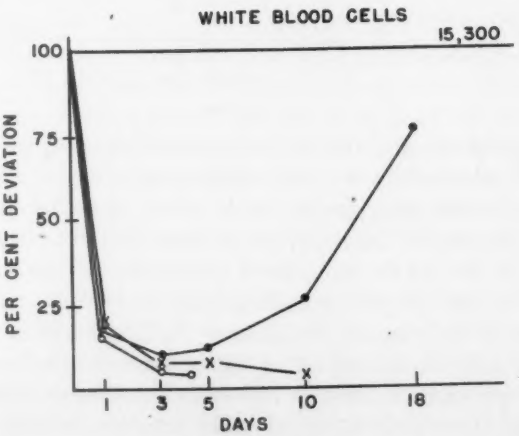
from the 3rd to the 5th day following exposure, and was roughly proportional to the amount of exposure. The depletion of platelets continued in group II to the 10th day. Group I, however, showed some



Text-figure 3.

indication of a rise in the platelet count after the 5th day, but the total number of platelets had not reached a normal level by the 18th day, the last on which observations were possible.

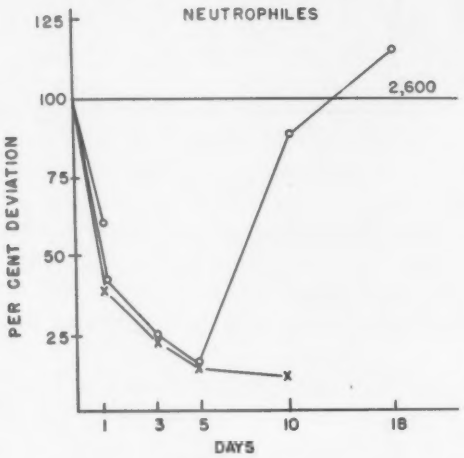
White Blood Cells. Since the great majority of white cells in the



Text-figure 4.

peripheral blood of the mouse are lymphocytes, fluctuations in the total white cell count largely reflect changes in the lymphocyte levels. Within 24 hours after bombardment, all three groups showed a striking depletion of the total white cells in the peripheral blood, to about 20 per cent of the normal value as shown in Text-figure 4. In group II the total white count continued to fall to the 10th post-treatment day, and group III likewise showed a continued drop to the 4th post-treatment day. These were the last days on which observations were possible in these two groups. Group I with the longest survival period showed a continued fall in the total white count on the 3rd day, after which there was a slow rise to 75 per cent of the normal value on the 18th day.

Neutrophils. Within 24 hours after treatment, the total neutrophil count of all three groups fell sharply (Text-fig. 5). This fall contin-



Text-figure 5.

ued in group II until the 10th post-treatment day, when the lowest point was reached, 12 per cent of the normal value. On the 5th day the average neutrophil count in group I was at its lowest level, 15 per cent of normal, but thereafter the neutrophil count of this group rose sharply, so that on the 18th day it was higher than normal.

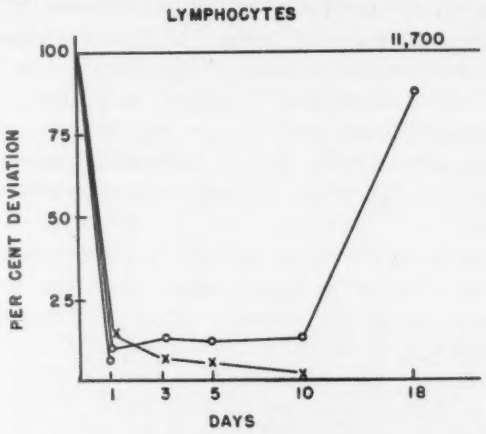
Lymphocytes. A prompt and sharp fall in the total lymphocytes occurred in all three groups as indicated in Text-figure 6. The first observation after treatment, at 24 hours, showed lymphocyte levels of 10 to 15 per cent of normal. The total lymphocyte count in group I was about 15 per cent of normal on the 10th day, but the next observation on the 18th day revealed a marked rise to a level approximating normal. In group II, however, the total lymphocytes continued to fall

to the 10th day when they were less than 5 per cent of normal. In group III the total white cell count was so low after 24 hours that differential counts could not be conducted and the lymphocyte level could, therefore, not be determined.

Tissue Alteration

In studying the various histologic sections, special attention was directed to the bone marrow, lymph nodes, and spleen (Figs. 1 to 7).

Bone Marrow. Within 24 hours following exposure there was a noticeable depletion of all cellular elements comprising the bone mar-



Text-figure 6.

row with the possible exception of megakaryocytes (Fig. 2). These changes occurred in all three groups of mice, but were most marked in group III. At 3 days marked hypoplasia of the bone marrow was noted in a majority of animals; in some cases large lakes of red blood cells had replaced the young forms usually present. Here and there islands of distorted cells and scattered megakaryocytes could still be seen. On the 5th day the maximum degree of aplasia occurred in both groups I and II, nearly all blast forms having disappeared. Thereafter, group I animals showed rapid regeneration (Fig. 3) so that by the 10th day they had bone marrow of normal cellularity, with distinct hyperplasia of the marrow on the 18th day. In group II, however, there was only slight evidence of regeneration on the 10th day, the last day on which observations were possible. In general terms the period of hypoplasia and aplasia lasted for about 10 days.

Lymph Nodes. At 24 hours after irradiation, animals in all three groups showed evidence of lymph node damage. This was character-

ized by foci of nuclear pyknosis and fragmentation in both the primary and secondary nodules where there was evidence of active phagocytosis in the many macrophages which had replaced them (Figs. 5 and 6). Necrosis and macrophages were present also in the medullary cords. In some of the smaller nodes the primary and secondary nodules had disappeared completely. There was distinct loss of cellularity in animals receiving higher doses, with interstitial edema, marked vascular congestion, and fibrous proliferation as a prominent feature. Reparative processes were evident on the third and fifth days with clearance of the cellular débris and cell regeneration as indicated by active mitoses (Fig. 7). On the 18th day the reparative activity was practically complete, the nodes in group I at this time showing definite secondary nodules, dense masses of lymphocytes in the primary nodules, and fully differentiated medullary cords. In summary, exposure to the deuteron beam caused early and marked cellular necrosis in lymph nodes, with loss of cellularity, followed by active macrophagic removal of necrotic débris and ultimate restoration of the anatomical configuration.

Spleen. The changes noted in the splenic tissue were on the whole similar to those observed in lymph nodes except that they appeared to be of longer duration, with recovery taking place less rapidly. Early cellular necrosis in both the red and white pulp was accompanied by a macrophage response with clearing of the necrotic débris. There was distinct loss of cellularity on the 1st and 3rd days with reduction in volume of both the splenic follicles and the red pulp. The latter was markedly congested and contained many extravasated red cells while the former had lost their outer sharp boundary of lymphocytes. Megakaryocytes also participated in this reduction, paralleling the decreased cellularity of the surrounding tissue. Considerable hemosiderin was present in all parts of the spleen as early as 24 hours after exposure and continued in group I to the 18th day. Repair proceeded slowly, with restoration of the volume and cellularity of the splenic follicles and red pulp. Occasional foci of extramedullary hematopoiesis were noted. Jacobson, Marks, Gaston, Robson, and Zirkle[8] have made a thorough study of the rôle of the spleen in hematopoiesis and radiation injury.

Discussion

The experiments here reported were undertaken preliminary to an investigation of the effect of the deuteron beam on transplantable malignant tumors. Necessary limitations on the use of the cyclotron for biologic studies made it impossible to accumulate sufficient data for evaluation by statistical methods. However, the changes observed so

closely paralleled known effects of roentgen and neutron irradiation that the need for precise statistical analysis was largely obviated.

It was anticipated that the changes produced by the deuteron beam on the peripheral blood and hemopoietic organs of the white mouse would closely parallel the alterations known to occur following other forms of penetrating irradiation. At all levels of exposure there was a prompt fall in the total white count, in the total number of neutrophils, and in the total lymphocyte count, and a slightly delayed reduction in the platelet count. Bone marrow became hypoplastic; lymph nodes showed atrophy and loss of lymphocytes; spleens showed cellular depletion, focal necrosis, and hemosiderin deposits. In animals that survived the observation period of 2½ weeks there was gradual restitution to normal peripheral blood values, and a restoration of the hemopoietic tissues to morphologic normality. The changes were quantitatively proportional to the roentgen equivalent doses, animals receiving the highest dosage responding most severely. The degree of reaction, both in the peripheral blood and in the tissues, was essentially similar to the reaction reported by others following exposure to equivalent levels of other penetrating ionizing radiations.

Summary

White mice were exposed to a single whole body irradiation by the deuteron beam of the 184 inch cyclotron in doses of 3.8×10^4 ergs/gm., 7.5×10^4 ergs/gm., and 14.9×10^4 ergs/gm.

Studies of the peripheral blood showed a prompt and significant reduction in the total white count, and in the absolute number of neutrophils and lymphocytes. A slightly delayed but marked reduction in platelets also occurred. Surviving animals which received the smallest exposure showed a return of these blood elements to normal levels by the 18th day.

Following exposure to 190 mev. deuterons, severe aplasia was noted in bone marrow. Lymph nodes and spleen showed focal cellular necrosis, macrophage proliferation, and loss of cellular constituents.

The blood and tissue changes after whole body exposure to the deuteron beam were proportional to the roentgen equivalent doses, and similar to the changes known to occur following other types of ionizing irradiation.

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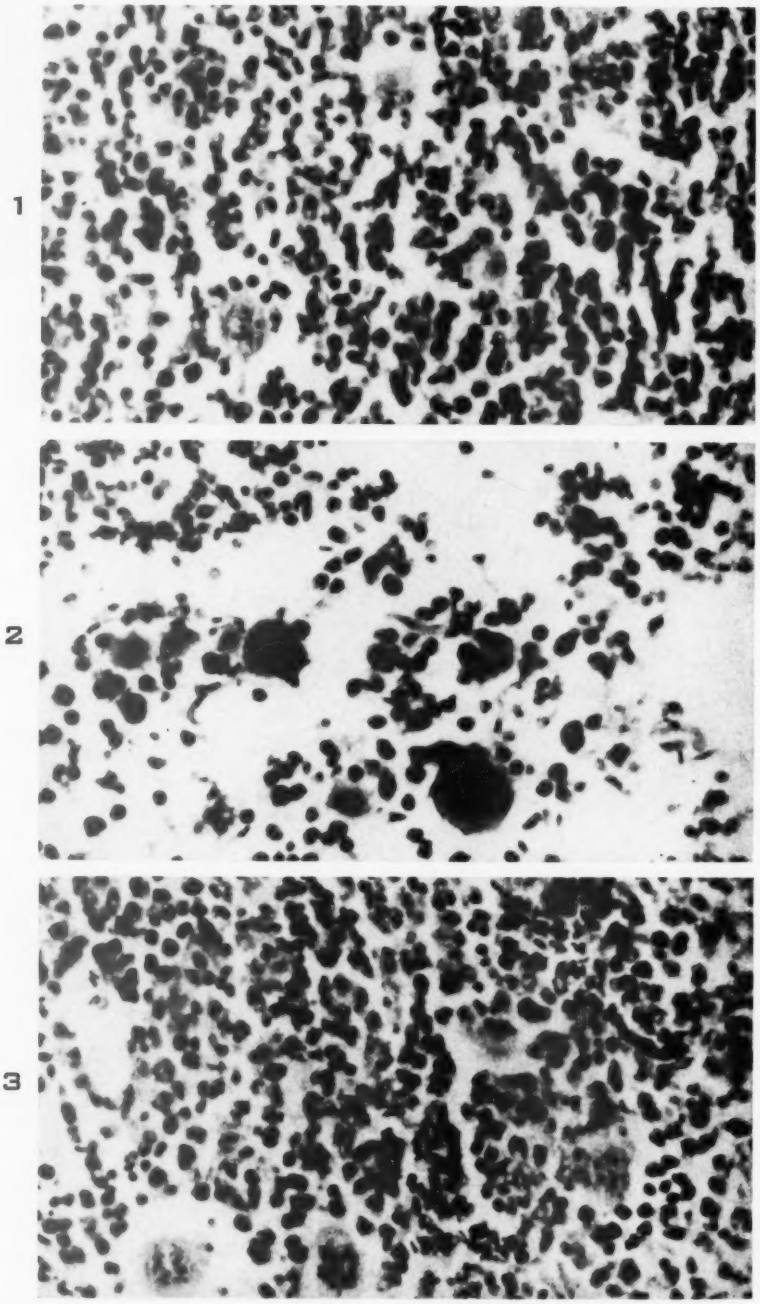
DESCRIPTION OF PLATES

PLATE 4

FIG. 1. Normal bone marrow. Hematoxylin and eosin stain. × 550.

FIG. 2. Bone marrow from mouse in group I, 24 hours after exposure. There is depletion of cellular elements with scattered megakaryocytes. Hematoxylin and eosin stain. × 550.

FIG. 3. Bone marrow from mouse in group I showing regeneration 10 days after bombardment. Hematoxylin and eosin stain. × 550.



Rosahn, Tobias, and Lawrence Single Exposure to 190 mev. Deuterons

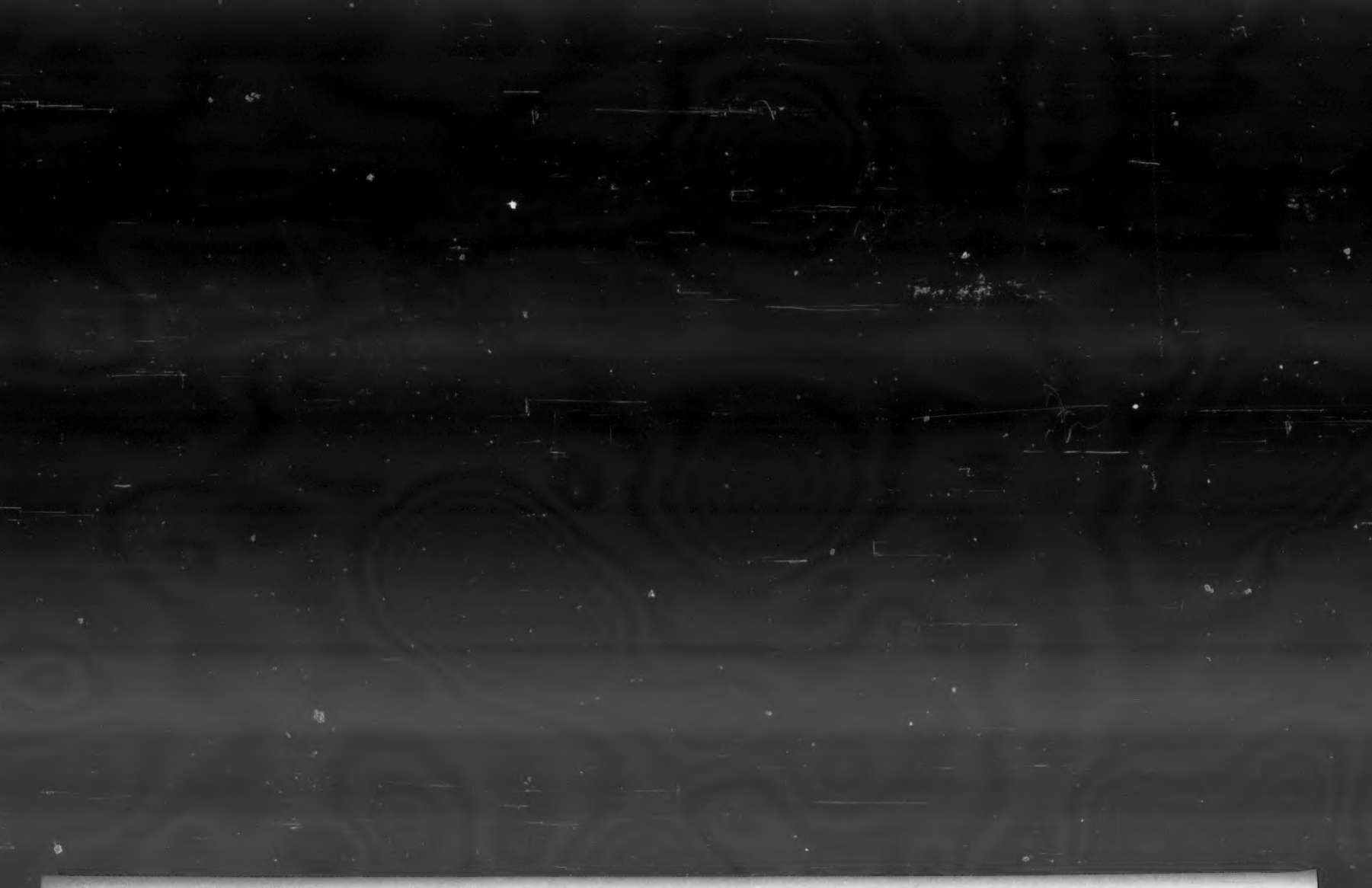
PLATE 5

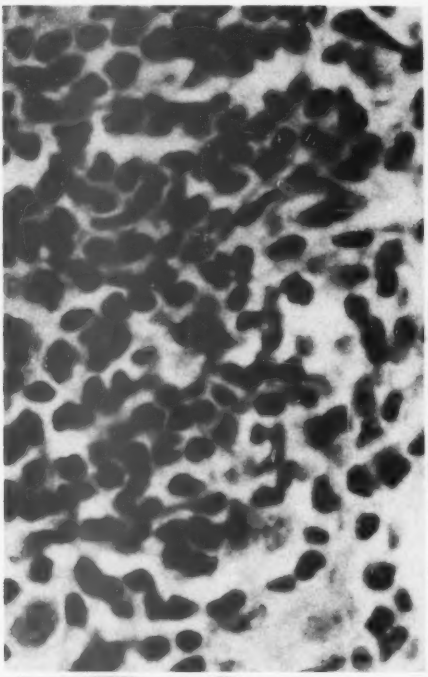
FIG. 4. Normal lymph node. Hematoxylin and eosin stain. × 1200.

FIG. 5. Lymph node from animal in group I, 24 hours after bombardment. The lymphocytes are numerically reduced, and nuclear pyknosis is noted. Hematoxylin and eosin stain. × 550.

FIG. 6. Lymph node from animal in group III, 24 hours after bombardment, showing nuclear pyknosis and fragmentation. Hematoxylin and eosin stain. × 1200.

FIG. 7. Lymph node from animal in group I, 10 days after bombardment. Regeneration with clearance of cellular débris. Hematoxylin and eosin stain. × 550.





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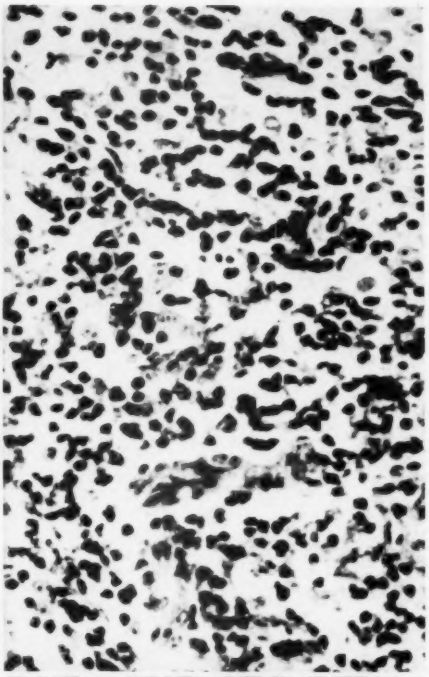

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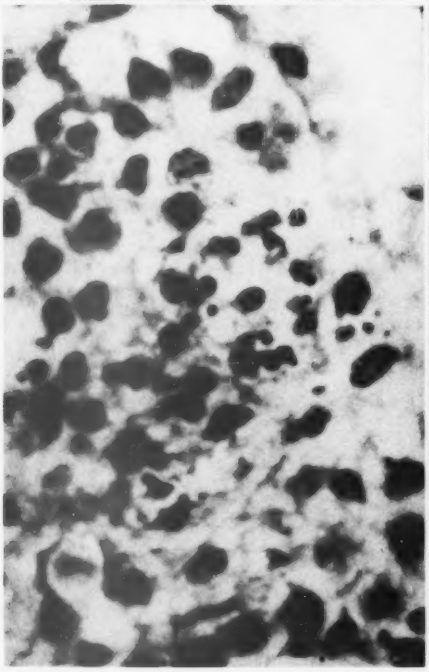

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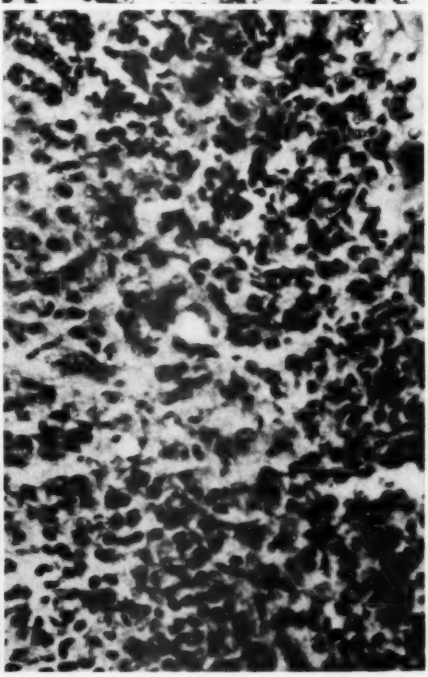

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Rosahn, Tobias, and Lawrence

Single Exposure to 190 mev. Deuterons

ASPIRATION OF GASTRIC CONTENTS
An Experimental Study*

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Whenever the pulmonary reaction resulting from aspiration of gastric secretions, or, more commonly, of gastric contents, is studied—whether the technics employed be those of the clinician or the pathologist—a discussion invariably follows as to the relative importance of the components of the aspirated material in the production of the pulmonary lesion. The studies comprising the basis for this report were carried out in an attempt to evaluate the part played by enzymes, pH, bacteria, and type of ingested food. It is easy to understand the difficulties involved in such a study if one considers the variations in quantity of free and combined hydrochloric and other acids, pepsin, two different mucins, other organic compounds, and significant amounts of sodium, potassium, calcium, bicarbonate, and phosphate—all constituents of the normal gastric secretion. Couple these complexities with the innumerable ingested foodstuffs at various stages of digestion and the variety of accessible organisms and it is little wonder that there is room for debate with regard to the relative importance of these variables in the picture of aspiration pneumonia.

The initial phase of the study, done by Adelson and myself,[1] dealt with the pH. Varying amounts of hydrochloric acid solution at differing pH values were introduced into the lungs of rabbits by a needle inserted between the cartilaginous rings of the trachea. It was found that above pH 2.4 the intensity of tissue response no longer paralleled the pH or amount of acid introduced but rather simulated the results of injections of water in control animals. In pH ranges lower than pH 1.5 the parallelism also was lost, a maximal response being produced in a given area with any hydrochloric acid solution with a pH value of less than pH 1.5. Filtered and unfiltered human gastric secretions correlated well with the hydrochloric acid solutions with regard to tissue reaction in various pH ranges and doses. Thus, gastric secretion of pH 1.5 evoked a marked tissue response as did hydrochloric acid solution at pH 1.5 when given in the same amounts. The response of the tissues to hydrochloric acid solution was predominantly a polymorphonuclear leukocytic infiltration, but in the animals injected with gastric secretion, after the initial polymorphonuclear response in the first 24 to 48 hours, the cellular exudate was mononuclear with many macrophages derived from the alveolar walls. If the gastric secretion

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was filtered before injection, the whole inflammatory response was less pronounced, of shorter duration, and mononuclear in type with little evidence of phagocytosis.

From this phase of the work the only pertinent conclusion that could be supported was that the degree of tissue response paralleled the pH and volume of the injected acid solution between pH 2.4 and 1.5. This is about the pH range of normal fasting gastric secretion, although the parietal cell secretion may have a pH considerably lower.[2] These values serve as a base line for more applicable data but actually give little information concerning the aspiration of gastric content as opposed to simple gastric secretion; it is the former with which we deal in aspiration pneumonia. For this reason the following experiments were carried out.

Experimental Procedure

Four separate meals (dairy, meat, vegetable, alcohol) were eaten on successive days by a healthy subject whose fasting gastric secretion had a pH of 1.5 on four isolated tests. Each of these meals was eaten when the subject was in a fasting state and the stomach was aspirated 1 to 2 hours after the meal to obtain partially digested material. One-half of each specimen was filtered through no. 4 filter paper and the pH of each ascertained using a Beckman pH meter. Anaerobic, aerobic, and fungus cultures were obtained on each of the specimens. By the use of gelatin digestion the possible trypsin content of each specimen was determined. Using Mett's tubes,[3] the pepsin activity of samples of the gastric content was determined; care was exercised in these procedures to adjust the pH of the system with a suitable buffer. Young adult rabbits of healthy stock, weighing about 2.5 kg. each, were stunned with light chloroform anesthesia and the trachea exposed. A no. 15 needle was inserted between the tracheal rings and a 5 cc. aliquot of one of the described specimens injected at the level of the carina. Usually the respiratory movements were sufficient to cause the specimen to drop regularly from the wide bore of the needle so that no force was used in the injections. Five animals were injected with each solution. In addition, 5 rabbits were injected with 15 mg. of pepsin in 5 cc. of hydrochloric acid-phosphate buffer solution, pH 2.5, and a control group of 5 rabbits injected with 5 cc. of the buffer solution. Fifteen mg. of pepsin was chosen since that amount produced the same amount of pepsin activity in the Mett's tubes as the unfiltered dairy sample which gave the highest pepsin activity value of the aspirated samples. Thus a total of 50 animals was used. One from each group was sacrificed on the 2nd, 5th, 9th, 14th, and 21st days by

a blow to the neck followed by immediate removal of the lungs to prevent acute congestion and edema. The pleural surface of a lobe was seared and the lungs of the 2, 5, and 9 day rabbits cultured aerobically, anaerobically, and for fungi on Sabouraud's media. The tissues were fixed in 10 per cent neutral formalin solution. Three or more sections were prepared from each animal for hematoxylin and eosin staining.

Results

Dairy Diet

The meal of the dairy diet consisted of one raw egg in chocolate milk shake and one slice of white loaf bread well spread with mayonnaise.

Unfiltered. In the 2-day animals which received the unfiltered sample there was a mixed exudate, predominantly polymorphonuclear but with many macrophages, particularly about foreign brown material which did not contain hemosiderin. This material was not doubly refractile when viewed with polarized light nor did it take the Sudan IV fat stain. At 5 days a miliary granulomatous response was present. On examination at higher magnification, each of these foci of reaction closely simulated a hard tubercle with epithelioid cells surrounded by macrophages and an outer rim of lymphocytes, other mononuclear cells, and a few polymorphonuclear leukocytes. In the centers of such reaction sites were particles or aggregates of light brown material of about 40 μ in size. Some of these were in macrophages; others were engulfed by giant cells of the foreign body type. No necrosis was present. In the 9-day animals this type of reaction was not present in the alveoli but some of the brown material was present in the bronchioles in conjunction with an extensive exudate of degenerating polymorphonuclear cells and young and old macrophages. The 14- and 21-day animals showed only thickening of the alveolar septa with young macrophages. The alveolar spaces and divisions of the bronchial tree were normal. The foreign material was not present and no granulomata were seen in these animals.

Filtered. The 2- and 5-day animals which received the filtered sample showed diminution of polymorphonuclear exudative response with a few monocytes and small amounts of edema in the alveolar spaces. The microscopic picture of the lungs of the remaining 3 animals in this group was normal. None of the brown particles were encountered in any of the animals.

Meat Diet

The meat diet consisted of one-half pound of top grade fried hamburger with catsup.

Unfiltered. At the end of 2 days after introduction of the unfiltered sample there was an extensive exudate of old and degenerating polymorphonuclear cells with an equal number of young and old macrophages filling the alveoli and bronchioles. The alveolar septa had a fibrous appearance as did the exudate. Multinucleated macrophages and a few Langhans' giant cells were present. The 5-day animal showed an exudative response almost entirely composed of monocytes with large numbers of macrophages filling the alveolar spaces and septa, but not present in the bronchioles. Focal areas resembling hard tubercles were present and such granulomatous nodules increased in number as the alveolar macrophagic exudate decreased. These were present in the 21-day animal. Particles of striated muscle could be recognized in the earlier animals and homogeneous eosinophilic material, not necrotic exudate, was present in the centers of the granulomata in the older lesions.

Filtered. The earliest animal in this group receiving the filtered sample had a mild polymorphonuclear and monocytic exudate in the alveolar spaces. The remainder of the animals had diffuse thickening of the alveolar septa with monocytes, predominantly young macrophages, attached to the septa. There was no exudate in the alveolar spaces.

Vegetable Diet

Two cups of cooked mixed vegetables including lima beans, peas, celery, carrots, and canned pear halves comprised the vegetable diet.

Unfiltered. The 2-day animals which received the unfiltered sample showed an extensive exudate of polymorphonuclear neutrophils and swollen macrophages. Foreign cellular material was present in the bronchioles and there was an obliterative bronchiolitis. The succeeding animals had focal areas of granulomatous inflammatory reaction about vegetable cellular débris and there was general thickening of the septa with monocytes. These miliary areas of granulomatous reaction with foreign body giant cells were present in the 21-day animals.

Filtered. The findings in the animals receiving the filtered sample were uniform. All had a slight thickening of the alveolar septa with monocytes, predominantly young, undetached, alveolar macrophages. The 2-day rabbit had in addition an interstitial polymorphonuclear response with a few polymorphonuclear leukocytes and macrophages in edema fluid in the alveoli. No definite areas of granulomatous reaction were present.

Alcohol Diet

The alcohol diet consisted of 1 qt. of beer and 2 oz. of blended whiskey.

Unfiltered and Filtered. The severity of the reaction produced by the unfiltered material was slightly more than the filtered but the type of reaction was the same and therefore the changes in both groups will be described together. The 2-day animals had an extensive exudate. Many old and degenerating polymorphonuclear leukocytes were present, but the predominating cells were young and old macrophages. There were focal areas of necrotic exudate. There was extensive infiltration of the interstices as well as the alveolar spaces by the reacting cells. In the 5-day animals the exudate of the alveoli and interstices had a loose, fibrous appearance and many plump fibroblasts could be seen among the macrophages. Marked proliferation of the alveolar epithelium was present in many areas. In the remaining 3 animals of each of these groups the process gradually became quiescent so that the 21-day animals had only slight thickening of the septa with monocytes. There was no increased fibrous tissue in the lungs of these animals and no alveolar reaction.

Buffered Pepsin Solution and Buffer Control

The tissues from the groups receiving buffered pepsin solution and the buffer control showed the same type of reaction. In the animals receiving the pepsin the response was slightly more pronounced. There was an initial edema and a few polymorphonuclear leukocytes were present in the alveolar spaces. At the end of 48 hours only a slight interstitial thickening by monocytes remained and this persisted in a diminished degree in all animals of the pepsin-injected group and until the 14th day of the buffer control group. There was no evidence of parenchymal necrosis or digestion.

Analysis of Data

Enzyme Studies

None of the aspirated samples produced gelatin digestion, indicating the absence of trypsin activity. The frequent presence of bile in gastric aspiration and in vomitus prompted this determination. It is realized that the pH of gastric contents would prohibit its action were trypsin present, but the neutralizing effect of the initial edema resulting from introduction of gastric content into the lungs could conceivably reactivate the enzyme. This did not occur *in vitro* after proper buffering to a suitable pH.

The determination of pepsin activity of the samples of gastric content is recorded in Table I. These all are in the range of normal. Fifteen mg. of pepsin USP XII in 5 cc. of a hydrochloric acid-phosphate buffer solution adjusted to pH 2.5 gave *in vitro* results com-

TABLE I

Characteristics of the Injected Solutions and the Subsequent Cultures from the Lungs

Gastric contents	pH	Pepsin activity	Trypsin activity	Cultures			
				Control	2 days	5 days	9 days
Dairy diet unfiltered	3.8	400	0	Lactobacilli *Bacillus subtilis*	No growth	Micrococci	*Staphylococcus aureus*
Dairy diet filtered	3.8	64	0	No growth	No growth	*E. coli*	Lactobacilli
Meat diet unfiltered	3.5	231	0	Lactobacilli	Lactobacilli	No growth	Lactobacilli
Meat diet filtered	3.4	36	0	Lactobacilli	Lactobacilli	Lactobacilli *B. subtilis*	Lactobacilli
Vegetable diet unfiltered	2.7	324	0	*Serratia marcescens*	Lactobacilli	*Rhizopus nigricans*	*E. coli*
Vegetable diet filtered	2.7	295	0	*Serratia marcescens*	*Escherichia coli*	Lactobacilli *B. subtilis*	Lactobacilli Enterococci
Alcohol diet unfiltered	1.8	334	0	Penicillium	No growth	Micrococci Diphtheroids	Lactobacilli
Alcohol diet filtered	1.8	295	0	No growth	*E. coli* *B. subtilis*	Lactobacilli *B. subtilis*	No growth
Pepsin in acid solution	2.5	368	0	No growth	No growth	Lactobacilli	No growth
Aqueous HCl acid solution	1.5			No growth	No growth	Micrococci	No growth

parable to the unfiltered dairy solution, which had the highest value for pepsin activity of any of the aspirated material. It should be noted in passing that some of the pH values of the gastric contents were above the optimum range for pepsin activity. This fact was considered in determining the pepsin activities and suitable buffers used. Lack of significant tissue alteration from injection of buffered pepsin solution leaves considerable doubt that this enzyme is of significance in the production of aspiration pneumonia. This is further emphasized when one considers the tremendous initial outpouring of edema fluid by the lungs when gastric contents are introduced. The neutralizing effect of this fluid, if only moderate, would place the resulting pH in a range that would be incompatible with significant pepsin activity. It seems best to eliminate serious consideration of pepsin activity from our thinking in dealing with aspiration pneumonia.

pH

The correlation of the pH of hydrochloric acid solutions and of gastric secretions in their effectiveness in producing pulmonary parenchymal alterations has been mentioned. The effective pH range of similar volumes was found to be below pH 2.5. Aqueous acid solutions or gastric secretions of higher pH produced approximately the same tissue reaction as isotonic saline solution.

It can be seen from Table I that the pH of all of the solutions, except the alcoholic, fell above the effective range for significant tissue change if such alteration was to depend in a large part on pH. Certainly it can be seen that there was no correlation of tissue response with pH on injection of the aspirated dairy, vegetable, or meat meals. Indeed, the aspirated meal of highest pH, unfiltered dairy, pH 3.78, elicited a more severe response on injection than did the unfiltered vegetable material, pH 2.7. Within the same diet group there was no correlation, unfiltered dairy meal of pH 3.78 producing a severe reaction in contrast to the tissue response to filtered dairy material of pH 3.78. That the ability of the two alcoholic solutions to produce an almost identical tissue response was in large part dependent on their pH, can be seen on comparison of the tissues from these animals with those from rabbits injected with hydrochloric acid solution or gastric secretion of the same pH range. This, of course, does not mean that the alcohol itself does not have an injurious effect when introduced endotracheally into the lungs of a rabbit.

The fact seems well established that the pH of aspirated gastric content is not important in the production of aspiration pneumonia unless it is below pH 2.5. I am unaware of data regarding the pH

of numerous specimens of gastric content, but 3 of the 4 specimens recorded here had pH values above the effective range.

Bacteriologic Findings

The opportunity for a variety of microorganisms to be introduced into the lungs by aspiration of gastric content is apparent. There is no doubt that the acidity of the normal gastric secretions, and to a lesser degree of the gastric contents, tends to destroy numbers of bacteria and in general maintains relative sterility. Nevertheless, organisms with the protective coating of food, those existing in the stomach and enmeshed in the gastric contents, and the numerous indigenous forms in the mouth and pharynx, including the fusospirochetal group, become of more moment when mechanically placed in the relatively sterile lower respiratory tract—which occurs when gastric contents are aspirated.

The results of control cultures on the aspirated gastric contents before injection and of the anaerobic, aerobic, and fungus cultures of the lungs taken at necropsy are to be found in Table I. Several of the organisms are obviously contaminants. A few colonies of the frankly pathogenic *Staphylococcus aureus* and the enterococci whose pathogenic capabilities would probably come to light under these experimental conditions were present in animals sacrificed at 9 days. The tissue response in each of the animals was granulomatous and could not be construed as representing a response to these organisms. The micrococci cultured from other animals were members of saprophytic species.

Lactobacilli were cultured with considerable frequency from animals of each group. The anaerobic and aerobic forms of this aciduric organism are commonly present in the mouth, and transiently are parasitic members of the scant gastric flora. They are apparently innocuous, particularly with reference to the present discussion.

In the 50 rabbits the lesions produced by intratracheal introduction of gastric content and buffered pepsin solutions seem to be entirely independent of bacterial activity in their pathogenesis. The difficulties of producing extensive pulmonary damage by direct instillation of organisms into normal lungs are well documented.[4] That bacterial activity of the alimentary organisms, especially those of the mouth, can be of tremendous importance in producing pulmonary injury is well known, but predisposing conditions must exist. A good example of this is the complex of mouth organisms required to produce fusospirochetal pulmonary disease. Other factors which tend to provide fertile ground for bacterial activity will be dealt with presently.

It suffices to state that bacteria do not play a primary rôle in the production of aspiration pneumonia; when they do become a significant secondary part of the process, their success is dependent on peculiar properties of the organism or alteration of the host.

Aspirated Material

There are many reports of the tissue response to material introduced into the lungs. This literature deals primarily with the metals, bacteria, and oils. The fate of some of these substances has been established in part. The oils have received particular attention inasmuch as they are frequently accidentally introduced into the lung or gain entrance through therapeutic or diagnostic procedures.[5,6]

The reaction of the lung to the varied complex organic substances which gain entrance by aspiration of stomach contents depends, it seems, on the same principles as the reaction to other materials. Thus, the reaction is, for the most part, a function of the particulate size and shape of the introduced material and of its chemical properties and their ability to evoke tissue reaction. From the data presented it would appear that the lasting response to the vegetable, meat, and cocoa particles is in part dependent on their size, since they are fairly large and cannot be promptly removed. Particles which lodge in the bronchioles may, of course, produce obstruction to the peripheral portion of the lung. If incomplete, the obstruction may be completed by the ensuing bronchitis and bronchiolitis of which the exudate may become organized with the production of obliterative bronchial lesions in which the foreign particles are enmeshed.

While the aspirated particles may be of similar shape and size, and the tissue reaction of the same basic nature, the severity may vary. This is adequately seen in comparison of animals of the vegetable and dairy meal groups in which the cocoa of the chocolate milk as the stimulating agent invokes a far more extensive response than vegetable cells of similar size. Such variations would seem to depend on differences in the chemical composition of the materials. The injected meat particles appear to be more readily disorganized and dispersed by phagocytosis. Vegetable fiber particles were found in the lungs at 21 days. The ability of particles to be phagocytized, disintegrated, or removed from the tissue is an important factor in determining the type and extent of the reaction.[7]

Summary of Experimental Results

The acute phase of aspiration pneumonia in the rabbit consists of pulmonary edema of some degree, pulmonary congestion with hemor-

rhage and diapedesis of erythrocytes, de-epithelialization of the bronchial mucosa, and a neutrophilic cellular response.[8] Depending on the extent of the pulmonary edema, or bronchial obstruction, death may follow within 36 hours or the animal may survive. The animal may succumb of asphyxia in the absence of pronounced pulmonary edema if the airway is obstructed by a column of material moving to and fro with respiratory efforts and not effectively expelled externally nor allowed to reach the periphery of the lungs. This acute phase of the reaction may be due to the shock of the injected material. The reaction is certainly non-specific, being produced by any of the injected material, and simulates that described by Moon[9] in terminal pneumonia.

With aspirated solutions free of gross particles and with pH ranges from 2.5 to neutrality, and almost without regard to quantity under 25 cc., the acute phase of the reaction is all that is encountered. With acid solutions of less than pH 2.5 or solutions containing gross particles, the inflammatory reaction continues and its severity and basic nature depend on the physical and chemical properties of the injected material. Thus highly acid aqueous solutions produce a continuing granulocytic response in contrast to the granulomatous reaction of the foreign body type called forth by some macroparticles in less acid solutions. Even in the latter group the tissue reaction evoked by particles of comparable size varies in severity. This is perhaps dependent on differences in chemical properties of the particles. Such continuing responses terminate at 2 to 3 weeks if uncomplicated. This phase of the reaction is more specific for the material introduced.

In some of the animals there was a chronic organizing pneumonia but none showed gross abscesses with cavitation or the so-called pulmonary gangrene.

Certain Clinical Applications

Certain of the principles discussed are of definite clinical importance. Prevention of aspiration of gastric contents cannot be stressed too vehemently. Planned surgical procedure invariably presupposes an empty stomach and in certain emergency operations, as appendectomy, an empty stomach obtains as a natural result of the malady. The obstetric patient who is admitted for prompt delivery, the traumatic fracture case, the intoxicated patient with an epidural or subdural hematoma are the likely candidates for vomiting and aspiration of gastric contents. Traumatic and mechanical injuries requiring some anesthesia and surgery are frequent in intoxicated patients, who usually have a partially filled stomach. Their last meal is almost invariably

poorly chewed. Prevention of aspiration of gastric content in these patients simply involves emptying the stomach through a lavage tube. A small bore suction tube is of no practical value here since it leaves the large food particles.

If gastric contents are aspirated, the acute reaction may be fatal and steps to combat pulmonary edema, which may be profound, should be instituted. Fluid and especially particulate matter should be removed as completely as possible from the respiratory tract. In some instances this may suffice to establish an adequate airway. If, however, the distress of the patient is not rapidly relieved, regardless of the attendant's degree of certainty regarding the patency of the airway, bronchoscopy should be effected immediately. Only by this method can the chunk of frankfort sausage or beef stew be removed at the operating table rather than at the necropsy table.

After the acute phase there should be continued efforts to expel any macroparticles from the bronchial passage. Such particulate matter usually does not completely obstruct the airway but the inflammatory exudate that it calls forth leads to obstructive bronchiolitis followed by atelectasis and more serious pulmonary disease.

The pathogenesis of aspiration pneumonia is not dependent on bacteria and therefore it is not to be expected that antibiotics will be of primary therapeutic value. The use of these drugs is endorsed as a measure to prevent secondary infectious complications. Regardless of the administration of antibiotics, the tissue response to the inanimate aspirated particles is expected to persist until adequate disposition of such particles is effected.

Clinically, the two important phases of aspiration pneumonia are the immediate reaction in which pulmonary edema or bronchial obstruction may jeopardize the patient's life, and the delayed complications of chronic bronchial obstruction.

Conclusions

In rabbits, aspiration of gastric contents causes pulmonary edema, congestion, hemorrhage, de-epithelialization of bronchial mucosa, and neutrophilic cellular response in the acute phase. These changes are non-specific. After the initial response the tissues react to the aspirated macroparticulate matter by granulomatous inflammation. Occasionally the pH of the aspirated material is below pH 2.4 and the continued reaction is granulocytic. A combination of these two types of cellular response may exist.

The enzymes present in aspirated gastric content have little or no importance in the pathogenesis of aspiration pneumonia or its com-

plications. The few pathogenic organisms present in gastric contents are not related causally to aspiration pneumonia but may be of importance in complications thereof.

Pneumonia resulting from aspiration of gastric contents is primarily dependent on the physical and chemical properties of the aspirated macroparticles. Regardless of the composition of gastric content, if its pH is in a range lower than pH 2.4, aspiration of this material will produce pneumonia.

Such complications of aspiration pneumonia as abscesses, bronchiectasis, and so-called pulmonary gangrene result primarily from obstruction of the bronchial tree by aspirated material or by the exudative reaction caused by aspirated material.

The experimental data indicate the procedures of importance in the prevention and treatment of clinical aspiration pneumonia.

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DESCRIPTION OF PLATES

PLATE 6

FIG. 1, A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of unfiltered gastric contents from the meat diet. Of note are the muscle fibers in the bronchiole of the 2nd day animal (see Fig. 8). × 130.

FIG. 2, A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of unfiltered gastric contents from the dairy diet. There are particles of cocoa in the tissue (see Fig. 10). × 130.

FIG. 3, A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of unfiltered gastric contents from the vegetable diet. × 130.

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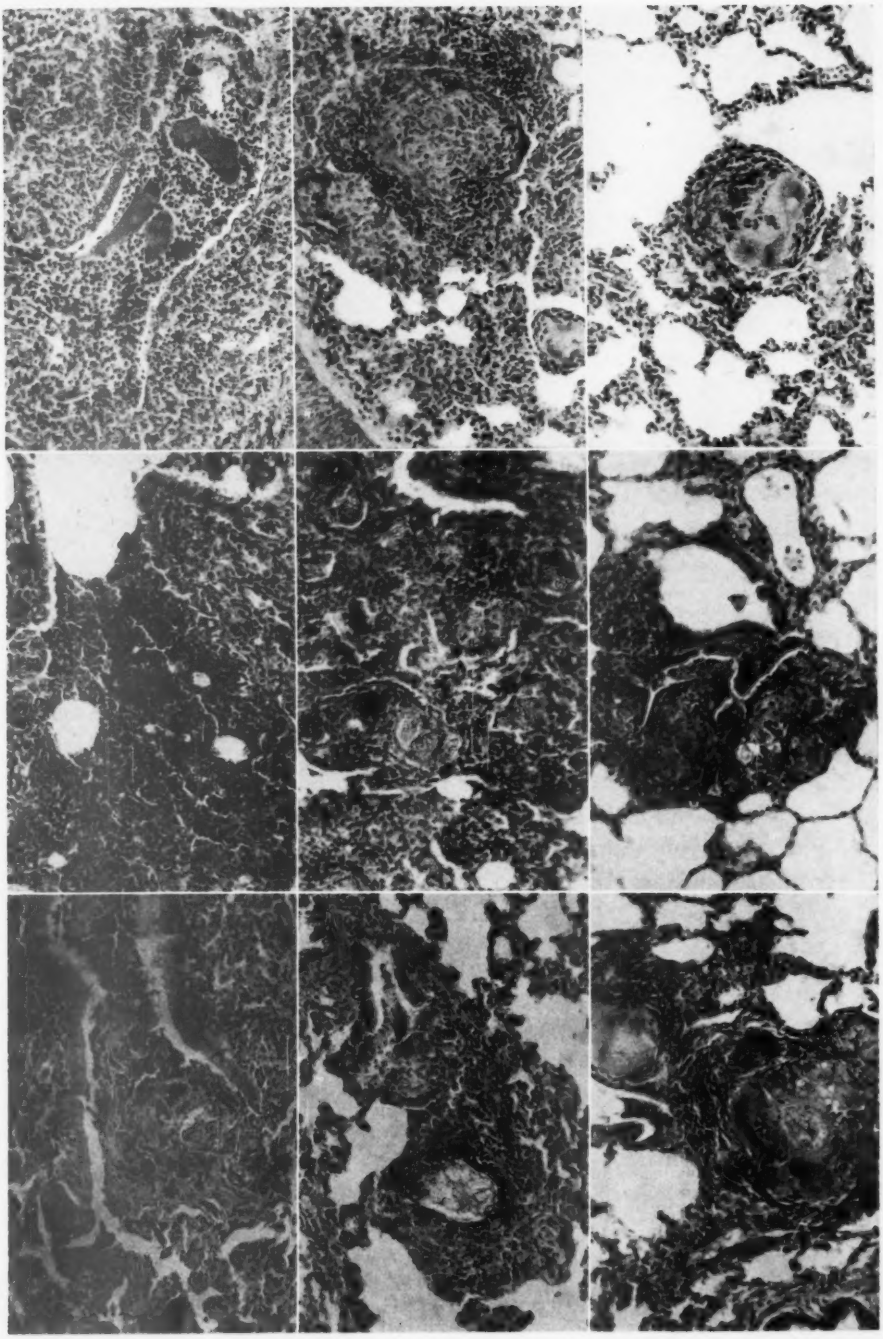
B,

2
B,

3
B,



Teabeaut

Aspiration of Gastric Contents

PLATE 7

FIG. 4. A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of filtered gastric contents from the dairy diet. The changes produced by injection of filtered gastric contents from the meat and vegetable diets were similar. × 130.

FIG. 5. A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of unfiltered gastric contents of pH 1.8 from the alcoholic diet. The changes produced by the injection of filtered gastric contents from the alcohol diet were identical. × 130.

FIG. 6. A, B, C. Pulmonary lesions on the 2nd, 9th, and 21st days after the injection of 5 cc. of aqueous hydrochloric acid solution, pH 1.5. × 130.

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A, B

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A, B

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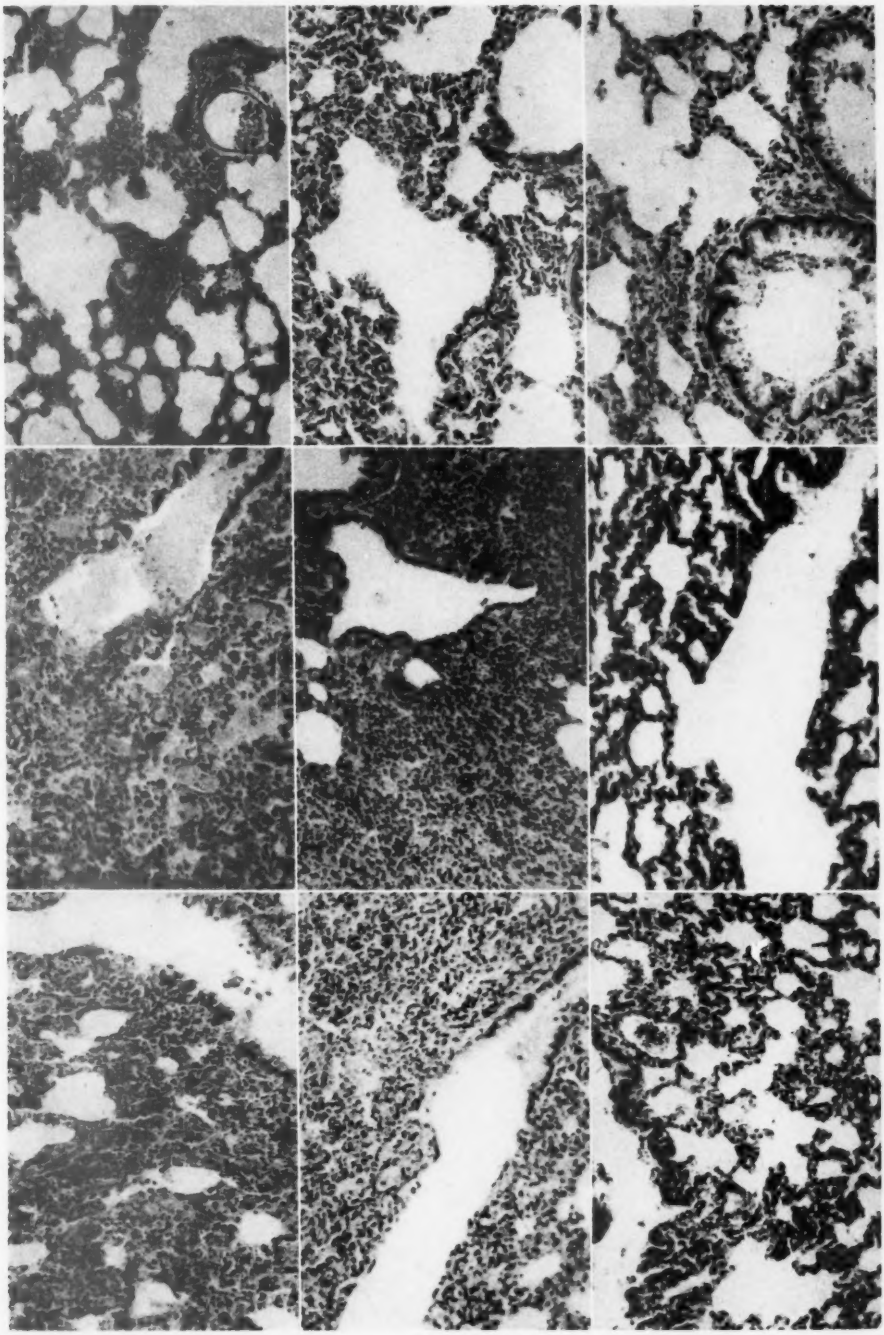
A, B



4
A, B, C

5
A, B, C

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A, B, C

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Aspiration of Gastric Contents

PLATE 8

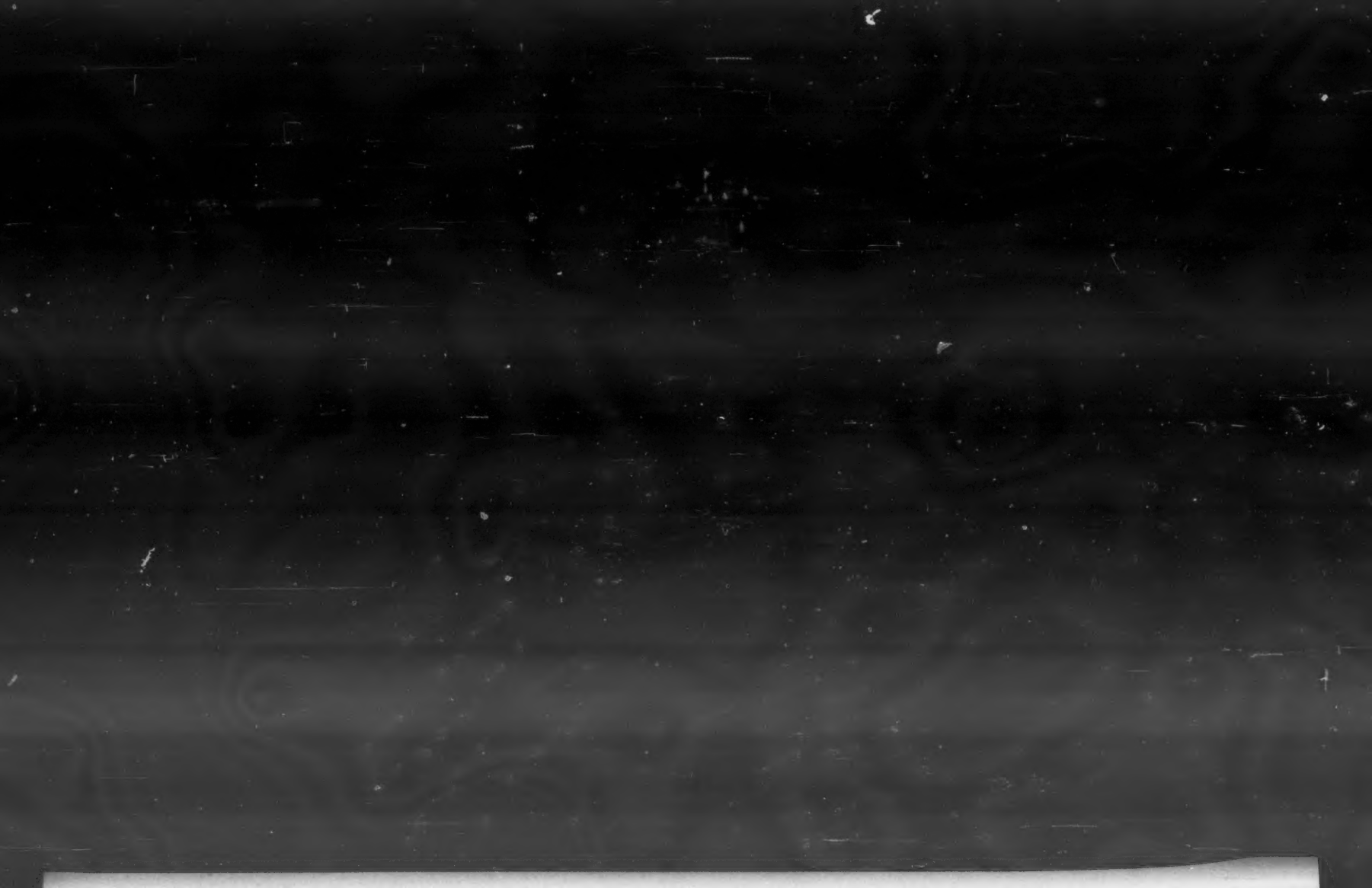
FIG. 7, A, B, C. Pulmonary tissue on the 2nd, 9th, and 21st days after the injection of a buffered acid solution containing 15 mg. of pepsin. × 130.

FIG. 8. A striated muscle fiber from the gastric contents 2 days after instillation into a bronchiole. × 500.

FIG. 9. Residual reaction about a striated muscle fiber 9 days after the injection of unfiltered gastric contents from the meat diet. × 500.

FIG. 10. Granulomatous reaction about cocoa particles 9 days after the injection of unfiltered gastric contents from the dairy diet. × 500.

FIG. 11. Residual reaction about vegetable cells 21 days after the injection of unfiltered gastric contents from the vegetable diet. × 500.



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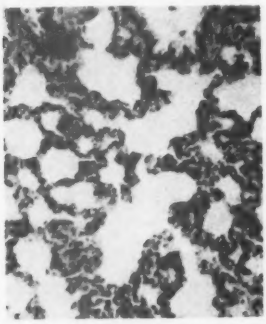
A, B

7
A, B, C



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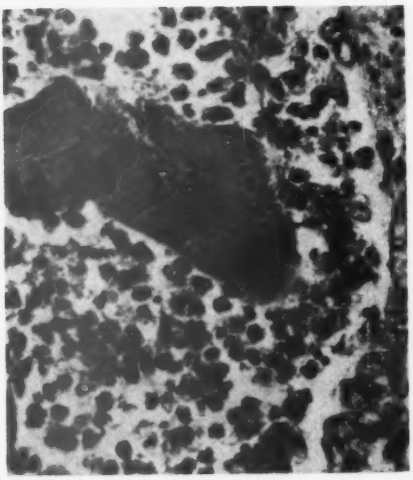

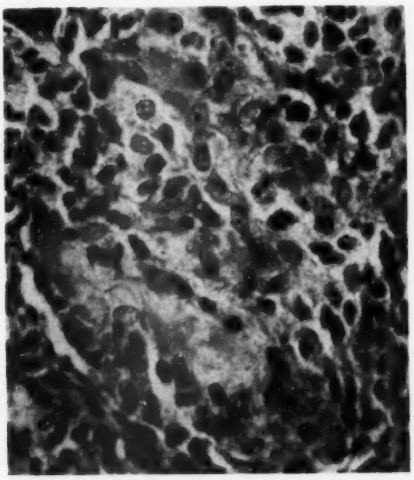

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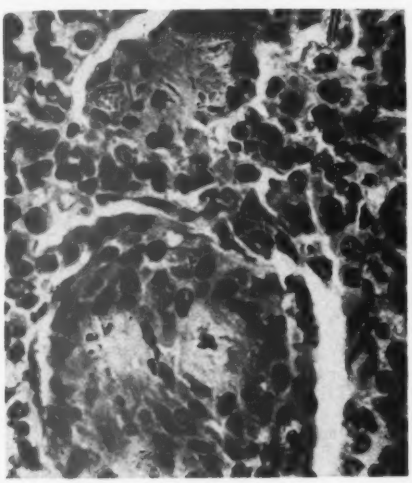

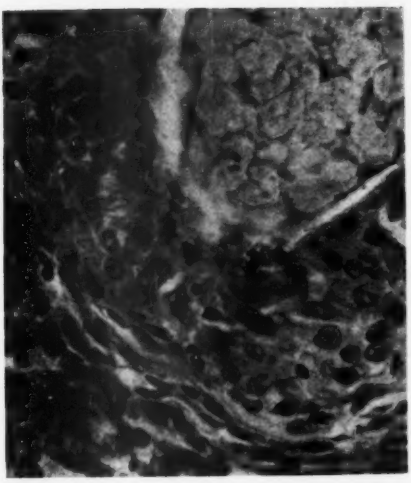

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Teabeaut

Aspiration of Gastric Contents

PULMONARY FIBROSIS SECONDARY TO PNEUMONIA*

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In the microscopic examination of sections of lung from necropsies performed at Thayer Hospital during the past 4 years, the occurrence of organization in pulmonary exudates seemed more common than anticipated on the basis of experience in general hospitals in the past. Casual survey of some of the material confirmed this impression, and a more thorough examination of all material was undertaken. Simple statistical analysis of the results showed a much higher incidence than was expected. When we attempted to compare this experience with those of others we were disappointed to find only rare statistical studies, although detailed descriptions of the pathogenesis and pathologic aspects were numerous. With respect to incidence, Symmers and Hoffman,[1] in a survey of 125 necropsies of lobar pneumonia prior to 1923, found organization in 3.2 per cent. Of 210 necropsies of lobar or "croupous" pneumonia, Lord[2] reported an incidence of 7.6 per cent of "organizing or indurative pneumonia," and Lauche[3] stated that with lobar pneumonia organization occurred in from 1 to 6 per cent and in a slightly higher percentage with patchy pnenumonia. In a clinical study, Musser and Norris[4] reported "delayed resolution" in 105 of 2,548 cases of pneumonia, but in a more recent clinical study Gleichman, Leder, and Zahn[5] observed delayed resolution (failure of resolution to occur within 30 days) in 52 of 198 cases. Our series is not comparable to any of these, since the material studied by us consisted of consecutive necropsies regardless of causes of death and was not confined to cases showing pneumonia.

MATERIAL

Sections from 307 complete necropsies (group I) collected over a 4-year period from mid-1946 to mid-1950 were available for study. A variable number of representative sections of lung were included in all cases. All individuals were adult and, with one exception, were male. The primary causes of death differed widely, but there was an unusually large proportion of malignant neoplastic diseases.

The sections of lung were examined microscopically for evidence of

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fibrosis. It was quickly apparent that pulmonary fibrosis of some type and degree was very common and that restriction of the study to organization of "pneumonic" exudate would be advantageous. Therefore, a number of common types of pulmonary fibrosis were arbitrarily excluded. For instance, cases of tuberculosis and other granulomatous diseases in which fibrosis is a natural sequel were not included. For the same reason chronic suppurative pulmonary diseases were omitted. Also excluded were numerous cases of primary or metastatic neoplasms of the lung and all examples of bronchogenic carcinoma and other thoracic tumors showing fibrosis attributable to radiation treatment. As nearly as possible the cases of fibrosis secondary to vascular disturbances (chronic passive congestion and infarction) were likewise omitted, but, among these, there were some showing lesions which were interpreted as additional organization of exudates. In brief, we attempted to confine our observations to instances of pulmonary fibrosis secondary to pneumonia, presumptively caused by exogenous infectious agents, in which resolution would be the usual expected outcome. As an exception to this generality, 7 cases of proliferative reaction of unknown cause occurring in the lungs of patients dying in renal failure were included.

In order to obtain a comparable series and with the idea that the increased use of antibiotics might have affected the incidence of organization, the files of the Department of Pathology of Vanderbilt University were consulted. One hundred necropsies from each of the years 1940 (group II) and 1930 (group III) were reviewed using the criteria and exceptions mentioned.

Gross Incidence

Organization in this restricted sense was encountered in 38 of the 307 necropsies in group I, or somewhat more than 12 per cent. Of the 100 cases in group II there were 7 instances of organization, and of the 100 cases in group III there were 5.

These relatively small groups do not permit of generalization or of deduction that organization is increasing, but the suggestion is made to stimulate similar comparisons.

Anatomical Lesions

The lesions of group I varied in size from lobar or greater to small microscopic areas. Only the extensive areas of carnification following lobar pneumonia were recognized as such in the gross; there were 3 such cases. Of the remaining 35, none was diagnosed until the microscopic sections were examined. Review of the gross descriptions

contributed no constant or peculiar findings, and small areas of organization were often hopelessly intermixed with fresh pneumonic exudates. Some few areas of gross size were set apart by circumscription and by firmness, suggesting metastatic tumor; and small patches of atelectasis were associated with underlying organization in several instances.

The microscopic characteristics varied also, but two fairly distinctive patterns were recognized: one in which intra-alveolar organization was predominant and one in which interstitial fibrosis was predominant. Although all cases fell into one of these patterns because of the major type of lesion, in many instances there was overlapping, and characteristics of both patterns could be seen in the same lung.

Primary intra-alveolar organization occurred in 16 cases in group I, one in group II (1940), and 3 in group III (1930). Fibroblastic invasion of the exudate with ultimate formation of strands of fibrous tissue in the alveoli and bronchioles was characteristic. Anatomically, these lesions frequently were found in a peribronchial or subpleural position, but, if large, resulted in a fleshy, elastic, carnified lung. The following cases illustrate this pattern:

Case 1

J. J. was a Negro male, 48 years old, who was admitted to Thayer Hospital on May 19 and died on June 22, 1947. Nine days prior to admission he had sudden onset of right pleuritic pain accompanied by cough, dyspnea, and fever. Several days before admission he was given sulfa tablets and penicillin by his local physician without improvement. The initial diagnosis was lobar pneumonia and empyema due to pneumococus type I. Purulent pericarditis was recognized 4 days later. He received penicillin from May 19 through June 22, sulfadiazine from May 30 to June 8, and had repeated chest and pericardial taps. His course was gradually downward.

The major anatomical diagnoses were chronic suppurative pericarditis, organizing pneumonia of the right lower lobe with localized empyema, and early left lower lobar pneumonia. The right lower lobe was densely adherent to the chest wall, was solid and heavy, and cut with the consistency of liver.

Microscopically, the alveolar sacs containing young connective tissue were not more than one-third of the total. They appeared in the majority at first glance because of partial collapse of the intervening sacs. The connective tissue bands and plugs anastomosed freely with neighboring masses, apparently through pre-existing defects (Fig. 1). The outer surface of the plugs often was covered partly or continuously with a layer of cells like those lining the alveolar membranes. The peripheral connective tissue of the plugs was dense while the central part was less compactly fibrous and contained many pigment-filled

macrophages and small round cells. These looser central portions also contained blood-filled capillaries and occasionally it was possible to see that these were derived from points of contiguity with alveolar walls. The air-containing alveoli were partially collapsed and contained numerous macrophages filled with hemosiderin. The walls were thickened but slightly or not at all, except as due to partial collapse. Polymorphonuclear leukocytes were numerous only in several bronchioles and immediately adjacent alveoli. There was early lobar consolidation in the left lung.

Comment. During this patient's 6-weeks illness, carnification of the right lower lobe and other complications occurred while he was receiving penicillin and sulfonamide therapy. Delayed and initially inadequate treatment probably contributed materially to the onset of the complications.

Case 2

E. R., a white male, 28 years old, was admitted on April 1 and died on May 8, 1947. Three hours prior to admission he suffered a self-inflicted gunshot wound of the head. The patient underwent operation immediately and had a very stormy postoperative course. He received penicillin intramuscularly, sulfadiazine intravenously and orally, and penicillin intrathecally from April 1 to 11. A clinical diagnosis of pneumonia was not made. During the next month the patient improved slowly from the effects of the wound but he died suddenly and unexpectedly.

The major anatomical diagnosis was gunshot wound of the head with penetration of the cerebral hemispheres, the right lateral ventricle, and corpus callosum. The lungs were generally air-bearing and essentially normal. There were several patches of subpleural atelectasis.

Microscopically, a number of terminal bronchioles and alveolar ducts contained solid fibrous plugs in which only a few centrally located macrophages persisted (Fig. 2). Cuboidal epithelium or low columnar epithelium covered some of the surface of the bronchiolar plugs. There was moderate infiltration of monocytes and lymphocytes in the surrounding bronchiolar and alveolar tissues.

Comment. Although a clinical diagnosis of pneumonia was never made, it is probable that this patient developed the infection during the critical postoperative period while on "prophylactic" penicillin and sulfonamide treatment. This minimal or abortive pneumonitis probably was of bacterial etiology. There was failure of resolution or incomplete resolution under intensive anti-bacterial therapy. The resulting organization of exudate was fully finished in 5 weeks.

Case 3

J. K., a white male, 54 years of age, was admitted on March 11 and expired on March 22, 1950. He had had pneumonia five times during the preceding 23 years

and "flu" 1 year before. He had frequent colds and was said to have had asthma for 10 years, although no treatment was necessary. About 2 weeks before entry he contracted a cold which was followed by "flu" and was characterized by fever, chills, diarrhea, and a cough which produced tenacious blood-streaked sputum. His local physician administered penicillin and sulfadiazine for "double pneumonia." After 3 days of medication the patient developed anuria and a measles-like eruption. The anuria lasted 2 days. All medication was discontinued 2 days before entry.

On admission there was evidence of extensive infiltration of the right lung, which later spread to the left. Breathing was deep and rapid but there was cyanosis of the hands and feet. Blood pressure was consistently low. The daily urine volume was 1,500 to 2,000 cc. but the specific gravity was about 1.013. There was retention of sodium without detectable edema, and the non-protein nitrogen levels were markedly elevated. Carbon dioxide combining power of the plasma was moderately lowered throughout the course. Repeated sputum cultures yielded only Proteus. The patient received streptomycin, aureomycin, penicillin, and chloromycetin singly and in combination without appreciable benefit.

The major anatomical diagnoses were confluent bronchopneumonia, unresolved; early portal cirrhosis; and nephrosis. There was epithelial regeneration in the damaged renal tubules, which was interpreted as a healing sulfonamide nephrosis.

The right lung weighed 1,470 gm. and the left, 1,060 gm. The right upper and lower lobes were firmly solid and other lobes showed patchy consolidation. The gross section was moist, deeply congested, and exuded frothy fluid. The smaller bronchi of both lungs contained yellow pus. There were poorly defined, soft, yellowish areas of necrosis in the right upper lobe.

Microscopic sections from the solid lobes presented a confused picture of septal thickening blended with intra-alveolar fibroplasia, and residual exudation of serum, fibrin, and leukocytes. The septa were thickened by edema, hyperemia, and infiltration of numbers of monocytes and small round cells (Fig. 3). In many areas there was active fibrosis in the septa, this being most notable at points of confluence and around the small vessels. Many alveoli were filled with loose, proliferating, fibrous tissue, often intermixed with fibrin containing monocytes, which fused with neighboring septa to obliterate normal structure in many small areas (Fig. 4). The majority of alveolar sacs were reduced in size and were commonly lined by a nearly continuous layer of small cells. The lumina contained serofibrinous material which was rich in monocytes and small round cells. Polymorphonuclear cells were very infrequent except in the scattered pus-filled bronchioles and adjacent alveoli. There were small irregular areas of acute necrosis in some of the sections, usually in relation to bronchioles.

Other areas of the lungs showed thin alveolar walls and alveolar sacs of normal size filled with seropurulent exudate. In one such area a lobule was acutely necrotic and contained masses of fungi identified as

Monilia. There was virtually no reaction to the presence of these organisms.

Comment. The lesions in this case illustrate intra-alveolar organization combined with interstitial fibrosis. It appears that the exudate being organized was a product of the initial infection which began about 25 days before death and which was excited by an organism sensitive to penicillin or sulfadiazine. The purulent, occasionally necrotizing, bronchiolitis and bronchopneumonia were attributed to superimposed infection by a strain of Proteus resistant to antibiotics. Infection by Monilia was considered to be terminal, but the background of therapy probably played an important part in its onset.

Interstitial fibrosis was the major lesion in 22 cases in group I, 6 cases in group II (1940), and 2 cases in group III (1930). In the stages studied, the lesions were fundamentally productive. While actual conversion of fibrinous exudate into fibrous tissue was a lesser feature, it did contribute to septal thickening, and the whole was therefore regarded as a "failure of resolution." Fibroplastic widening of the alveolar walls was an outstanding feature. The attendant alveolar exudate was composed of fibrin and was very dense, frequently taking the form of "hyaline membranes." Fibroblastic invasion of this material usually was apparent. The end result of the process was the formation of scars of varying size, usually subpleural or peribronchial. The following cases illustrate this pattern:

Case 4

M. W., a white male, 52 years old, was admitted on March 9 and died on March 17, 1948. The patient noted the onset of mild, cramping, abdominal pain 2½ days prior to admission and, 4 hours before, developed severe right lower quadrant pain. Emergency laparotomy revealed a perforated appendix and generalized peritonitis. His postoperative course was very stormy, and he received penicillin and sulfadiazine throughout his hospital stay. On the day before death a clinical diagnosis of pneumonitis and pleural effusion was made.

The major anatomical diagnoses were peritonitis, due to *Escherichia coli,* with secondary pelvic abscess, and lobular pneumonia of both lungs.

On microscopic examination some areas were relatively normal. In all sections, however, there were rather large and poorly defined areas of alveolar fibrosis which were usually in relation to bronchioles. The fibrosis varied from slight to marked and the alveolar sacs were correspondingly reduced in size (Fig. 5). Fibroplasia was active, so that the alveolar walls appeared cellular and there were moderate numbers of infiltrating monocytes and lymphocytes. The appearance of cellularity was exaggerated further by proliferation of alveolar lining cells

which often formed a nearly continuous membrane around the constricted sacs. In all the diseased areas there were many membranous deposits of dense fibrin lining alveolar ducts and sacs. Monocytes in small numbers were present in and about this material. Invasion of the fibrin by fibroblasts derived from alveolar walls was evident in some areas.

In several patches there was acute purulent exudation superimposed on the older disease. The fibrin deposits appeared less abundant and somewhat less dense in these patches, as if there were partial solution.

Comment. This patient was in good health until the onset of appendicitis and peritonitis. Assuming that pneumonia developed during the immediate postoperative period, it was of not more than 8 days' duration. His pulmonary complications developed in spite of intensive anti-bacterial therapy. The microscopic picture is quite different from that seen in cases 1 to 3 and more nearly resembles that described for viral pneumonia.

Case 5

J. B. was a white male, 41 years old, who was admitted on February 5 and expired on February 12, 1948. The patient had had malaise and intermittent low grade fever since 1945 and swelling of the abdomen, jaundice, and enlarged lymph nodes, since October, 1947. The past history was of interest in that the patient had pneumonia on three occasions from 1941 to 1945 while in the Navy. Details of these attacks were unknown. The clinical impression on admission to this hospital was Hodgkin's disease, which was confirmed by cervical node biopsy. He was given small doses of roentgen therapy over the abdomen and inguinal regions and supportive treatment but went rapidly downward. Penicillin was administered only during the last 2 days of life.

The major anatomical diagnosis was Hodgkin's disease of the abdominal and thoracic lymph nodes, the liver, spleen, and sternal bone marrow. The pulmonary parenchyma was not involved. Gross examination of the lungs showed only congestion of the lower lobes.

Microscopically, there were moderate numbers of "dust cells" and erythrocytes in many alveoli. Scattered bronchioles were loosely filled with fibrinopurulent exudate. Approaching the pleura, there was rather prominent peribronchial fibrosis, and the tissue was infiltrated with leukocytes of all kinds including moderate numbers of polymorphonuclear cells. The subpleural portion of several sections presented zones of varying width in which the alveolar walls were markedly thickened with fibrous tissue (Fig. 6). Some walls were ruptured, creating large, irregular air spaces. Other spaces of alveolar size were surrounded or constricted by wide fibrous walls. Scattered monocytes and lymphocytes were present, and the air spaces contained many macrophages. In one small area the air sacs contained a dense deposit of fibrin and

there was early organization of this material. Polymorphonuclear leukocytes were rarely seen in this area.

In another section a wider zone of loose, subpleural scarring was present (Fig. 7). Remnants of ducts incorporated in this scar retained their epithelial lining and there were many dilated non-muscular blood vessels. Slight fibroblastic activity was observed around an entering bronchiole and in some of the bordering alveolar walls.

Comment. The pulmonary lesions in this case seemed to be unrelated to the Hodgkin's disease. The peribronchial and subpleural scarring appeared to be a logical sequel to such a process as described in case 4. This patient gave a history of three bouts of pneumonia from 1941 to 1945, the details of which are not known, but these illnesses might well account for the remote pulmonary fibrosis discovered at necropsy.

Seven of the 22 cases of interstitial fibrosis differed somewhat from those described and deserve special comment because of common clinical and pathologic findings. All of them occurred in cases of *intrinsic disease of the kidneys terminating in uremia.* Pathologic features common to all were engorgement, edema, and leukocytic infiltrations of the alveolar walls combined with endothelial swelling and cellular proliferation. The alveoli usually contained exudate composed of serous fluid, blood, or compact fibrin, this sometimes taking the form of hyaline membranes. In addition, there were small foci of active organization, usually intraluminal but sometimes interstitial. The following cases illustrate these changes:

Case 6

C. C., a white male, 25 years of age, was admitted on December 23, 1947, and expired on February 6, 1948. In November, 1947, the patient had "flu," followed in 2 weeks by edema of the feet, legs, and face, and "smoky" urine. He remained under the care of his local physician until referred to this hospital. A clinical diagnosis of acute glomerulonephritis was made. The patient was described as being in uremia at the time of admission, and his blood non-protein nitrogen rose to 146 mg. per 100 cc. His course was progressively downward despite dietary and supportive therapy. On several occasions he developed pulmonary edema and congestive failure. Penicillin was administered during the first week of hospitalization and again during the last 2 days of his life.

The major anatomical diagnosis was progressive glomerulonephritis. There were also fibrinous pericarditis and myocardial hypertrophy. Grossly, the lungs showed splotchy bright red or dark red discoloration in the hilar areas shading to faint pink near the pleural surface.

Microscopically, congestive changes were severe but not uniform. Patchy or lobular distribution of the graver lesions usually was apparent. Alveolar walls were thickened by hyperemia, edema, and

endothelial swelling. Some capillaries were thrombosed. Leukocytes, predominantly polymorphonuclear, infiltrated the walls in some lobules and in these there was intra-alveolar hemorrhage. Intra-alveolar edema was not a feature.

In addition to these more acute lesions, there were changes which seemed older. Scattered throughout in random fashion were small groups of alveoli filled with compact fibrin containing only a few monocytes. Some such deposits appeared inert, perhaps by reason of shorter duration, whereas others were being organized *in situ* or incorporated in the alveolar wall (Fig. 8). The product of the former was a striking focus of endothelial and fibroblastic proliferation partially filling an alveolus, and, of the latter, a similar cellular focus expanding a septum. The over-all picture of cellularity of the diseased areas was enhanced by proliferation of alveolar lining cells.

Case 7

B. F., a white male, 56 years old, was admitted for the fourth time on October 28 and died on November 9, 1949. He was known to have had severe hypertension for more than 2 years and had experienced anginal pain, dyspnea, and headaches for about 2 years. For 8 days prior to admission he had symptoms of left ventricular failure. In this hospital he improved temporarily on digitalis, penicillin, and supportive therapy but then developed intractable pulmonary edema and expired. The non-protein nitrogen was elevated throughout his hospital stay and several days before death was 108 mg. per 100 cc.

The major anatomical diagnoses were arteriolar nephrosclerosis, cardiac hypertrophy, and generalized arteriosclerosis. Grossly, the left lung was largely atelectatic. The right was wet and boggy. The lower lobe was firmer and darker.

Microscopically, the lesions were congestive, exudative, and proliferative, combined in varying degrees. No particular distribution of lesions was apparent. Some areas were air-containing and only mildly hyperemic. Others were more congested and showed numerous thin, fibrinous membranes lining alveolar ducts and sacs. Several sections, of which one contained a portion of a medium bronchus and was thus identified as being from the hilar region, presented large areas of very compact solidarity. The air spaces were packed with fibrinous or serofibrinous exudate, often mixed with blood. Monocytes dominated the cellular response in most areas but there were many polymorphonuclear leukocytes in the serous exudate. There was acute purulent bronchiolitis in one section and polymorphonuclear cells were predominant in the exudate of neighboring alveoli.

In the solid area the alveolar walls were markedly thickened by congestion, edema, endothelial swelling, and infiltration of leukocytes.

The appearance of widening and cellularity of the septa was accentuated by proliferation of alveolar lining cells (Fig. 9). Scattered throughout were many small areas of organization, some almost confined to alveolar spaces and others, usually smaller, expanding alveolar walls. The actively organizing luminal plugs often were capped with hyperplastic alveolar lining cells.

Discussion

The volume of material studied in this series does not warrant a conclusion that pulmonary fibrosis secondary to pneumonia is increasing. But comparison with the similarly unselected cases from the years 1930 and 1940 seems to make the suggestion valid. Of possible factors contributing to such an increase, two are foremost: one pertaining to etiology, and one to antibacterial therapy.

There is a considerable body of opinion that the clinical picture of pneumonia has become altered during the past decade, primarily by an increase in atypical or viral pneumonia.[6-9] It has even been suggested that viral pneumonia might represent the underlying disease in most bacterial pneumonias (Francis[8]), including the pneumococcal type (Israel *et al.*[6]). The pulmonary lesions of primary atypical pneumonia are described as purulent bronchiolitis, mononuclear cell infiltration of bronchial and alveolar walls, and the formation of hyaline alveolar membranes (Golden[10]), a descriptive picture which is very similar to that of 1918 influenza (Goodpasture[11]). It is stated that organization of the intra-alveolar fibrinous membranes is "not infrequent" (Golden[10]) or as occurring in "the majority of cases" but not extensively (Parker, Jolliffe, and Finland[12]). The lesions in 15 of 22 cases of interstitial fibrosis in this series conformed in major respects to those of primary atypical pneumonia. Additionally, there are several showing active alveolar fibroplasia which closely resembled, except in extent, the acute diffuse fibrosis of Hamman and Rich,[13] a disease presumed by them to be of viral etiology. Also encountered were a number of more or less subpleural scars similar in structure to the non-specific apical caps studied by MacMillan[14] and thought to be due to mild or chronic disease, possibly viral. In none of our 15 cases in group I was a clinical diagnosis of viral pneumonia entertained. Cultural examinations were performed on a minority of the 15 and all yielded a mixture of organisms, predominantly of types not ordinarily expected to be pulmonary pathogens. Acute purulent bronchiolitis was superimposed on the older disease in some of these.

Intra-alveolar organization, which was the principal lesion in 16 of the 38 cases in group I, was presumed to be secondary to bacterial

pneumonia, although cultural evidence was seldom conclusive. Mixed flora were obtained from the sputum and throat cultures performed, including those from one case of classical lobar pneumonia. Prior antibacterial therapy had been administered in nearly every instance. The cultural data were generally disregarded because they conflicted with the clinical findings; but there is evidence to indicate that alteration of the flora by antibacterial treatment may have adversely affected natural resolution of pneumonic exudates due to pneumococci. Experimental pneumococcal pneumonia seldom organizes (Sale and Wood,[15] Gunn[16]), but organization is a prominent feature of experimental pneumonia due to Friedlander's bacillus. Pneumonia induced in dogs by a mixture of staphylococci and pneumococci was more prone to organize than pneumonia due to pneumococci alone (Wadsworth[17]).

Also, and perhaps more importantly, antibacterial agents, especially antibiotics, may alter the natural host response through the effect on the causative organism. Solution of fibrin has long been held to be a function of enzymes derived from polymorphonuclear leukocytes. Exudates which are rich in fibrin and poor in these leukocytes are more apt to organize. In our material there was notable paucity of polymorphonuclear cells in the involved areas of most cases, and the predominant cell was the monocyte. However, organization was in an advanced stage in some of these, so that there was little persistent cellular response of any kind. Experimental confirmation of this possibility is lacking, although Wood *et al.*[18-21] observed that macrophages were more important in the phagocytosis of pneumococci in experimentally infected animals treated with sulfapyridine than in those treated with antiserum.

An attempt to correlate the factor of time with respect to duration of disease and period of antibiotic therapy was not satisfactory. The date of onset of pneumonia could seldom be determined with reasonable certainty and many of the patients were on "prophylactic" penicillin treatment. However, it could be established from three of the records that organization was active within 7, 8, and 9 days after the onset of pneumonia. This is a distinctly shorter interval than the usually accepted 2-week period.[3,22]

The significance of uremia as a cause of "pneumonia" accompanied by fibroplasia is unknown. "Uremic edema" or "uremic pneumonia" has been referred to as an entity principally because of its peculiar central or hilar distribution as seen in the radiologic shadow. The few pathologic descriptions of the pulmonary disease[23,24] emphasize capillary congestion, thickening of the alveolar wall, and deposition of dense fibrin in alveoli and bronchioles. Organization of the fibrin gives rise

to the "bronchiolitis obliterans" of Ehrich and McIntosh.[25] The 7 cases of "uremic pneumonia" in this series conformed in most respects to the previous descriptions. We were impressed with the similarity of the microscopic lesion to those of the so-called rheumatic pneumonia.[26] The foci of organization were indistinguishable from the "Masson body."[27] All of the patients in our series had experienced one or more episodes of left ventricular failure, and it seems likely that this was the principal common feature.

Summary

Pulmonary fibrosis secondary to pneumonia was found in 38 (12 per cent) of 307 necropsies performed between 1946 and 1950. Comparable series of 100 cases from each of the years 1940 and 1930 showed 7 and 5 per cent, respectively. The evidence suggests that the use of antibacterial agents contributes to a rising incidence of organization of pulmonary exudates, and that small areas of fibrosis may be the consequence of viral pneumonia.

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[*Illustrations follow*]

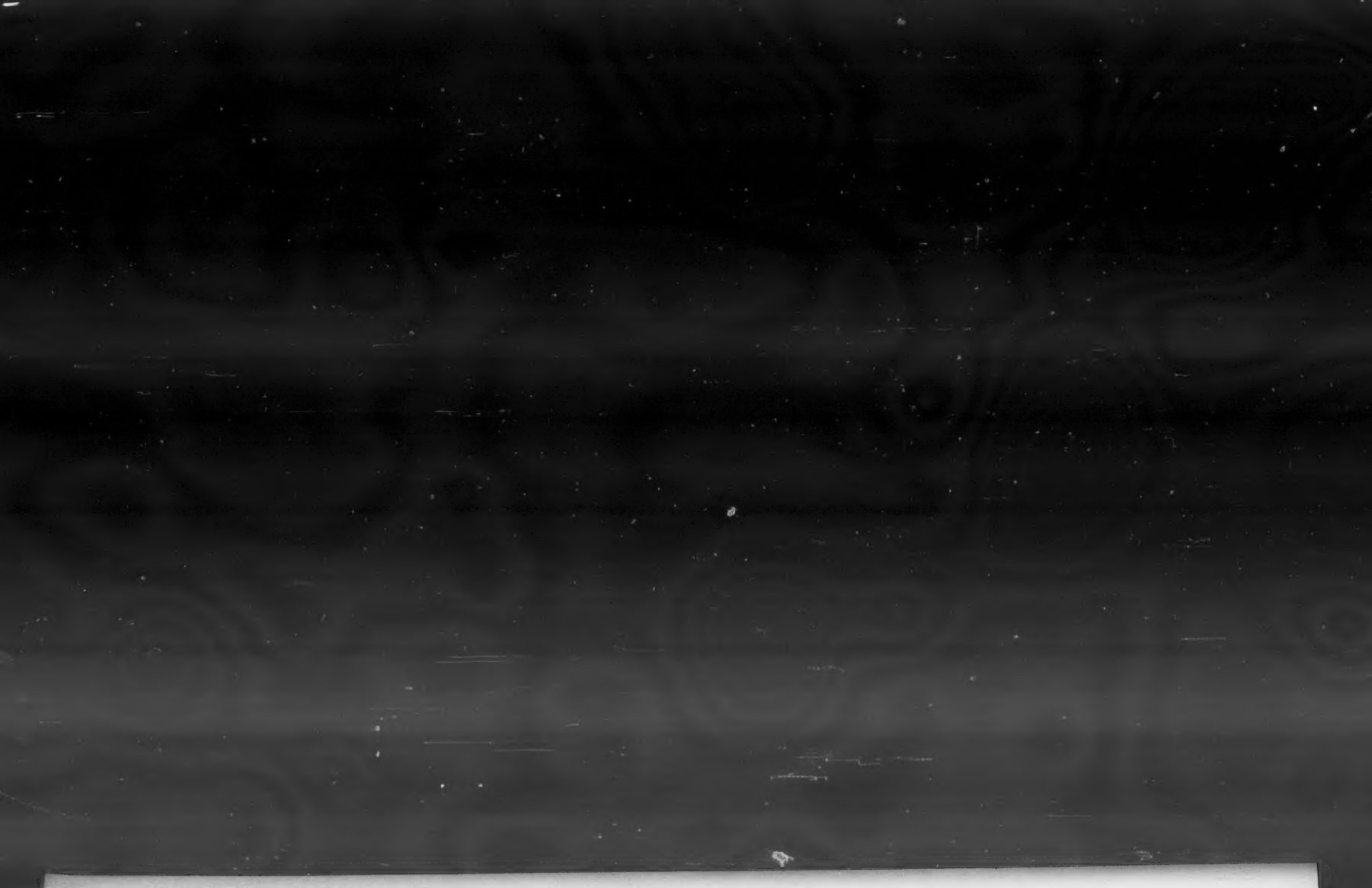
DESCRIPTION OF PLATES

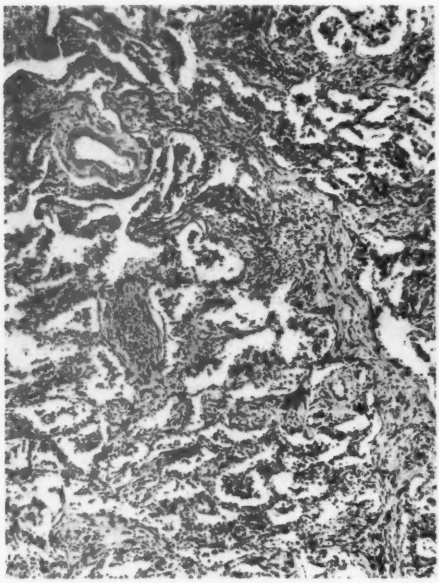
PLATE 9

FIG. 1. Case 1. Intraluminal organization secondary to pneumococcal lobar pneumonia. Anastomosing fibrous bands fill many alveoli. × 60.

FIG. 2. Case 2. Intraluminal organization in a terminal bronchiole about 5 weeks following pneumonia. The large fibrous plug is partially covered with cuboidal epithelium. × 60.

FIG. 3. Case 3. Combined intraluminal and interstitial organization about 25 days after the onset of pneumonia. × 40.





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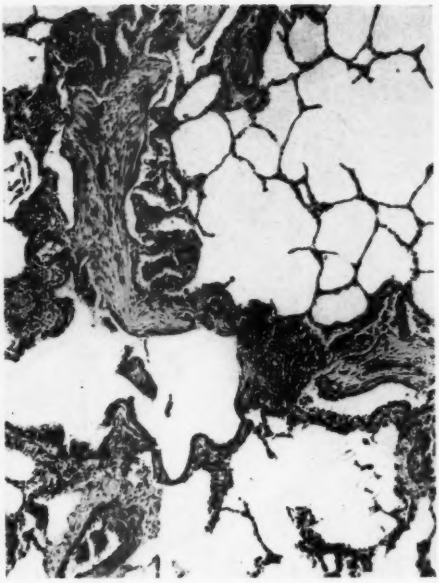

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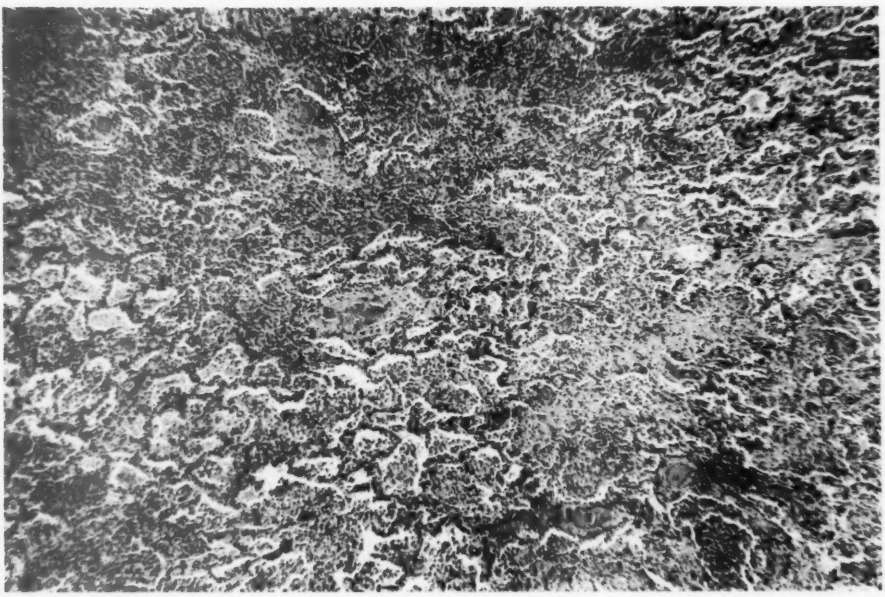

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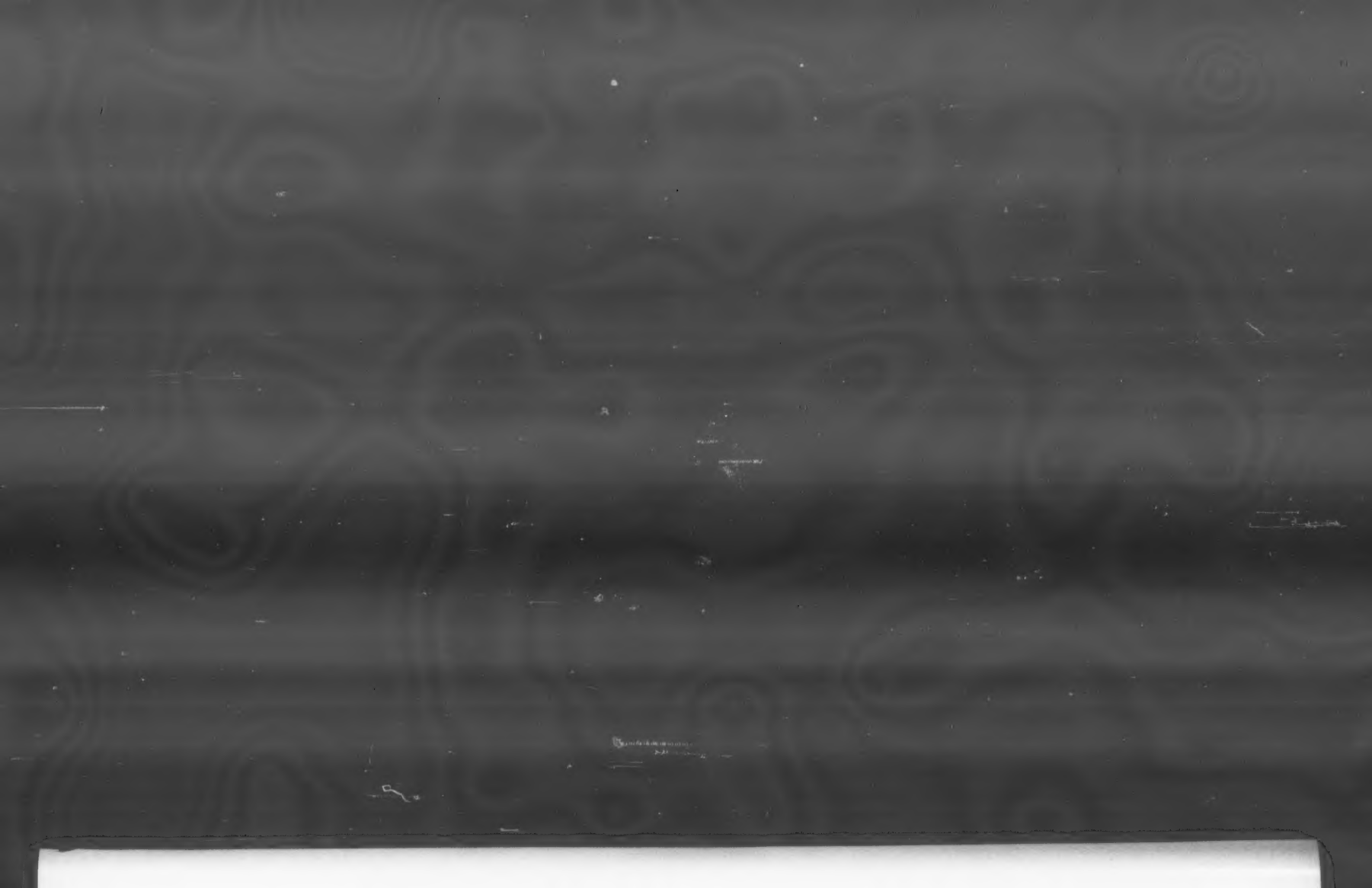
Auerbach, Mims, and Goodpasture Pulmonary Fibrosis Secondary to Pneumonia

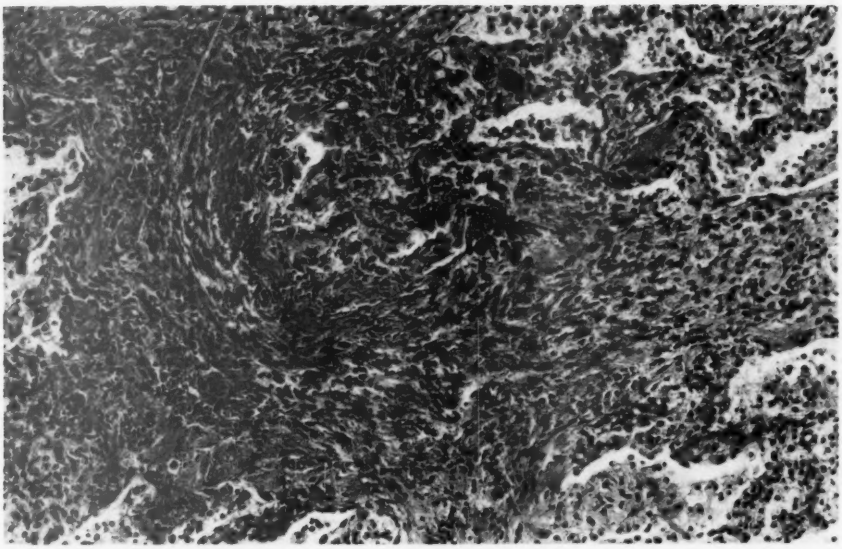
PLATE 10

FIG. 4. Case 3. Higher magnification of Figure 3, illustrating active fibrosis obliterating small groups of alveoli. The residual exudate is mononuclear. × 120.

FIG. 5. Case 4. Interstitial fibrosis, probably secondary to viral pneumonia. Membranous deposits of dense fibrin appear darker and are partly incorporated in the septal walls. × 60.

FIG. 6. Case 5. Interstitial fibrosis, possibly secondary to viral pneumonia. Intraluminal deposits of fibrin are more distinct. Mononuclear cells predominate in the reaction. × 60.





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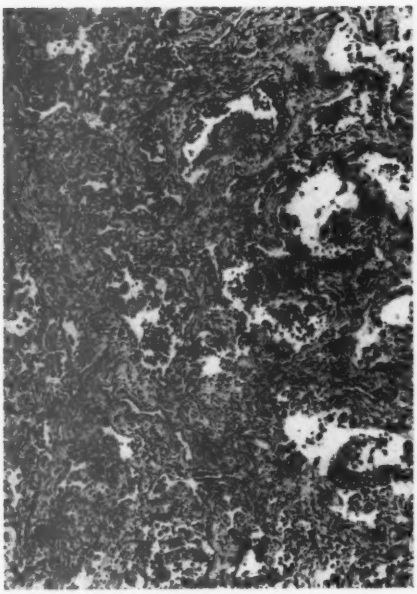

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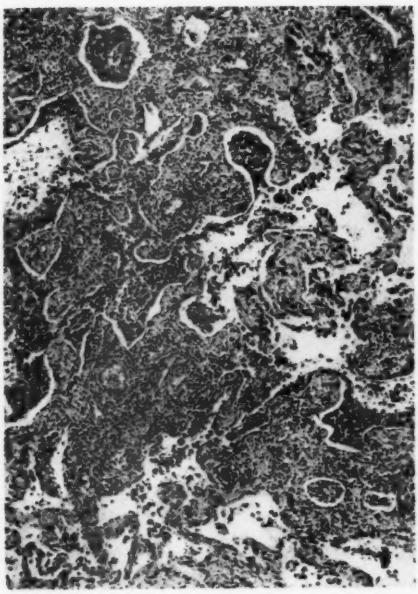

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Auerbach, Mims, and Goodpasture

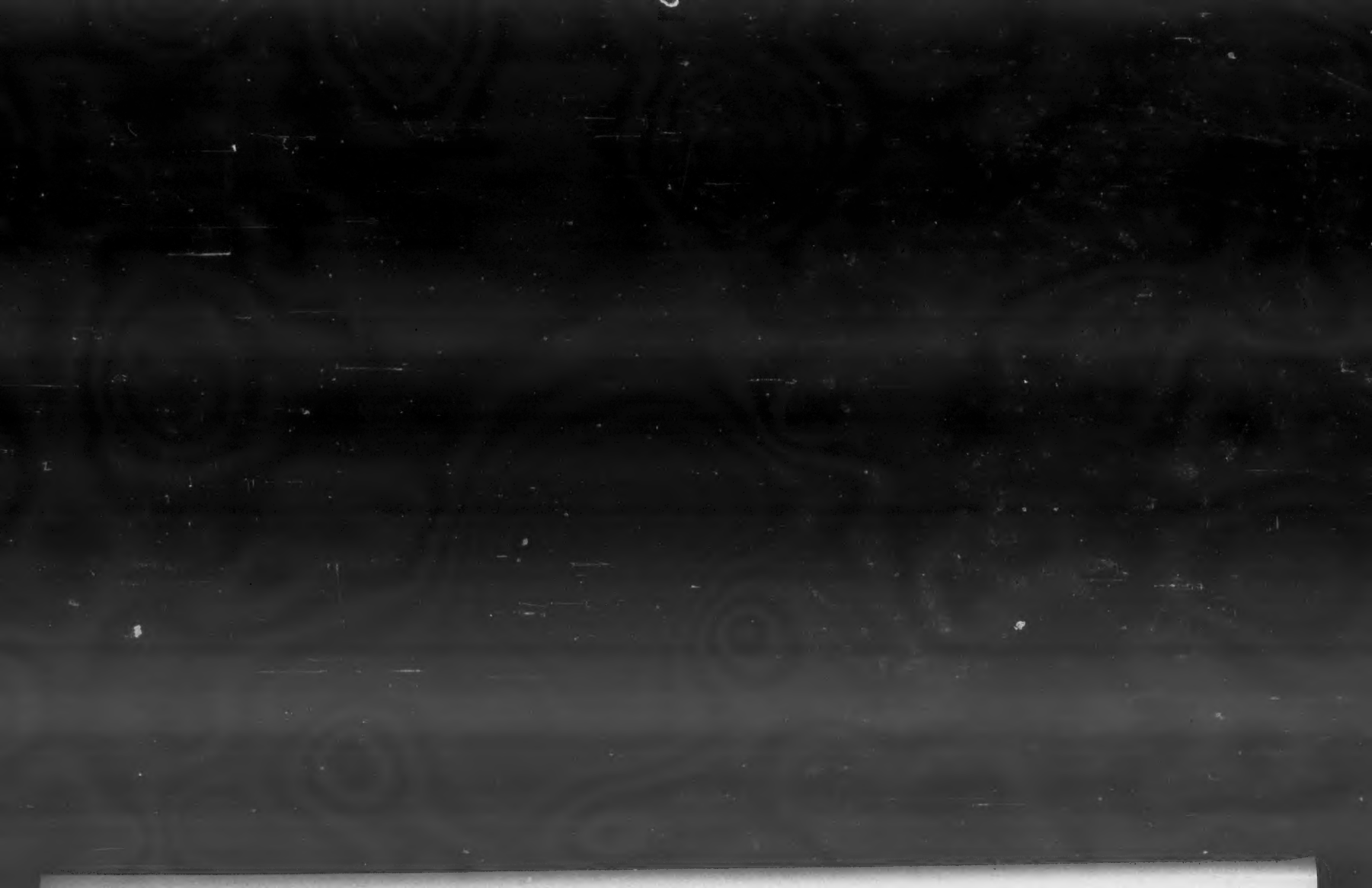
Pulmonary Fibrosis Secondary to Pneumonia

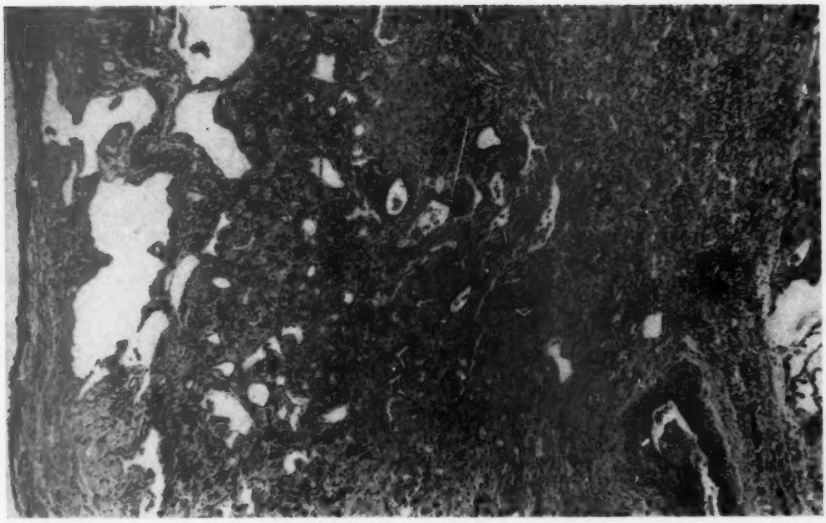
PLATE 11

FIG. 7. Case 5. Subpleural scar, possibly secondary to viral pneumonia. Persistent ducts lined by cuboidal epithelium are scattered through the mid-portion of the area. × 30.

FIG. 8. Case 6. Septal thickening and intra-alveolar organization in uremia. The large intraluminal plug is covered with cuboidal cells. Dense edema fluid in other sacs. × 100.

FIG. 9. Case 7. Septal fibrosis in uremia. Intraluminal plugs were present in other areas. × 100.





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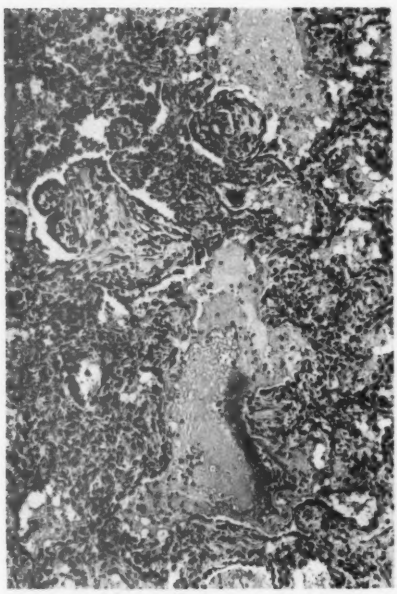

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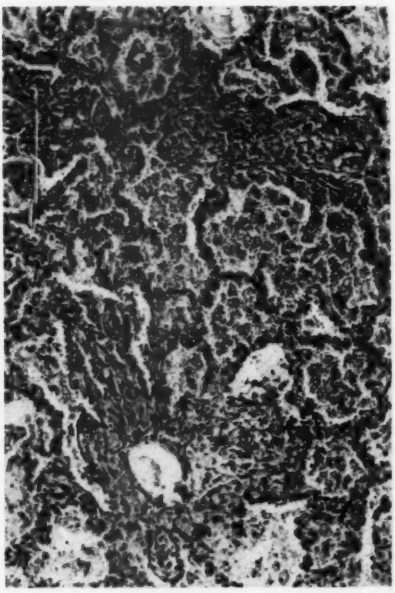

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Auerbach, Mims, and Goodpasture

Pulmonary Fibrosis Secondary to Pneumonia

THE BRONCHIAL ARTERIES

III. Structural Changes after Division of the Rat's Left Pulmonary Artery*

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Under conditions which produce impairment of pulmonary arterial blood flow, certain gross changes occur in the bronchial arteries of man and experimental animals.[1-6] These changes include an increase in size and seemingly in the number of the bronchial arteries and the development of demonstrable precapillary anastomoses between these vessels and branches of the pulmonary artery. With the exception of the report of Liebow and co-workers,[6] no systematic microscopic studies of the changes occurring in the bronchial arteries after ligation of a pulmonary artery have been made. With few exceptions,[7-10] the microscopic structure of the normal human bronchial arteries received little attention until the recent studies of Verloop.[11] This investigator has reported also a microscopic study of these vessels in the rat and in other rodents.[12]

The present experiments were undertaken for the purpose of studying the structural changes occurring in the bronchial arteries after division of the left pulmonary artery in the rat. That such a procedure is well tolerated by man[13-17] and the dog[2,3,6,18-20] has been demonstrated repeatedly. The experiments were planned in such a way as to give further information on the extent of the gross changes occurring in the bronchial arteries under these conditions and on the rapidity with which these changes may take place. Furthermore, it was hoped that some information could be obtained concerning the site of development of precapillary anastomoses between bronchial and pulmonary arterial systems.

Technic

Healthy male albino rats of the Wistar strain, each weighing 170 to 210 gm., were used. The animals were anesthetized with pentobarbital sodium (nembutal) given intraperitoneally in amounts equal to 25 mg. per kg. of body weight. An intratracheal tube of polythene was inserted into the trachea under direct vision through an otoscope.

* Abridgment of portion of thesis submitted by Dr. Ellis to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Surgery.

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Oxygen under positive pressure was delivered through the polythene tube in accordance with the method described by Porter and Small.[21]

The operative procedure consisted of opening into the left thoracic cavity, doubly ligating the left pulmonary artery, and dividing the vessel between ligatures. After the technic had been perfected, about 3 of every 5 animals survived. Some succumbed to pneumonia during the immediate and late postoperative periods. The rats were killed by a lethal dose of ether at postoperative intervals of 1 day and 3 days, at weekly intervals for 1 month, and at monthly intervals for 6 months. Three to 5 rats were available for study in each of these postoperative periods.

After death, a 10 per cent suspension of barium sulfate in water was injected at pressures of 100 to 110 mm. of mercury into the ascending aorta of 2 animals of each group. Thereby, the bronchial arteries were filled with the injection medium and could be recognized grossly and on microscopic examination. This suspension did not traverse the capillaries and was useful in demonstrating the presence of precapillary anastomoses with the pulmonary arterial system. Sections for microscopic examination were made through representative areas of the left lung of each rat and were stained with hematoxylin and eosin and with Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. Similar sections were made through the left lungs of a series of normal rats.

Corrosion casts were prepared from the lungs of 7 rats at postoperative intervals of 4 to 6 months. These casts were prepared by the injection of different colored solutions of vinyl acetate* or neoprene† into the aorta and bronchus with the lung *in situ*. After the injection, the lungs were removed and placed in concentrated hydrochloric acid for 24 hours and then washed in tap water. Control casts of normal rats' lungs also were prepared.

Results

Gross Changes

The left lung remained viable in all cases. It was somewhat decreased in size and was pale in comparison to the right lung. Vascular adhesions were encountered frequently between the thoracic wall and the lung at the thoracotomy site and between the lung and the mediastinal pleura and pericardium.

Detectable enlargement of the left bronchial arteries was apparent 3 days after operation. After 1 week there was further enlargement,

* Obtained from Ward's Natural Science Establishment, Inc., Rochester, N.Y.
† Obtained from American Anode, Inc., Akron, Ohio.

and collateral systemic vessels appeared traversing the left pulmonary ligament and running through adhesions and over the left bronchus and pulmonary vein. By the end of 2 weeks a tortuous collateral systemic blood supply was well established through the routes mentioned. The bronchial arteries themselves were tortuous and appeared to be dilated to three or four times their normal diameter when compared with the vessels of the right lung. In specimens examined 3 weeks and 1 month after operation these changes were slightly more pronounced. Figure 1 is a posterior view of a rat's lungs 3 weeks after division of the left pulmonary artery. The marked increase in size of the left bronchial arteries and the apparent increase in number of systemic blood vessels supplying the left lung are demonstrated.

Progressive changes in the left bronchial arteries after the first postoperative month were slight, and at the end of 6 months the bronchial arteries on the side of the divided pulmonary artery were approximately four or five times their normal diameter. A vinyl acetate cast demonstrating the tortuosity of these vessels as compared with those on the right is pictured in Figure 3.

The most rapid and striking changes in the left bronchial arteries occurred during the first few weeks after division of the left pulmonary artery. The most extensive changes usually were seen in rats surviving the longest. However, there was some individual variation in that some specimens at 4 months showed less extensive changes than others at 1 month.

Microscopic Changes

Normally two or three bronchial arteries are easily visible in the walls of the rat's larger bronchi on microscopic examination. Such a bronchus is seen in Figure 4. These vessels are formed for the most part by a medial layer composed of circular muscle, on the inner surface of which lies an internal elastic membrane which is more prominent in vessels close to the hilar region than in the more peripherally located branches (Fig. 5). The bronchial arteries are more difficult to visualize in the walls of the smaller bronchi than in the walls of the larger bronchi. Even after injection of a barium sulfate suspension into the aorta, they are not easily recognizable, for the injection medium does not readily penetrate to this zone. The larger pulmonary arteries on the other hand have a medial layer generously supplied with elastic fibers. The smaller branches of the pulmonary artery located more peripherally have an extremely thick medial muscle layer, a prominent internal elastic membrane, and a rather small lumen. The thick medial layer when seen in longitudinal section appears somewhat segmentally thickened.

On microscopic examination, the changes seen in the left bronchial arteries of the rat after division of the left pulmonary artery paralleled, in general, the gross changes already described. After 3 days, perceptible dilatation was encountered. By 3 weeks this dilatation was striking and was associated with tortuosity of the vessels and the presence of numerous vessels in the bronchial wall (Fig. 6). The increase in number of bronchial arteries in the left bronchial wall after left pulmonary arterial division was in part only apparent. Tortuous vessels cut several times in the same section gave an impression of an increase in number. On the other hand, vessels previously of the size of capillaries may have dilated and appeared as new vessels. In some cases an enlarged bronchial artery encroached on the lumen of a small bronchus (Fig. 7). The injection medium readily penetrated the dilated bronchial arteries around the smaller bronchi.

In uninjected specimens, the thickness of the bronchial arterial wall could be studied. Some increase in thickness was noted in one specimen 3 weeks after division of the left pulmonary artery. This thickening was encountered more consistently in specimens examined after longer postoperative intervals, and at the end of 5 months the medial layer was definitely thicker than normal and the muscle fibers were noticeably hypertrophied (Figs. 8 and 9). The internal elastic membrane was noted in some instances to have lost its linear character in cross section and to be composed of multiple, fine, elastic fibrils which penetrated the muscular media in places.

Uniform changes in the pulmonary arteries were not encountered and no significant abnormalities of the pulmonary parenchyma of any specimens were noted after division of the left pulmonary artery.

Anastomoses

Normally, the injection into the aorta of a 10 per cent suspension of barium sulfate fills only the bronchial arteries, indicating that no precapillary anastomoses with the pulmonary arterial system exist normally, or that, if they do exist, they are of insufficient caliber to receive the suspension of barium sulfate. Two weeks after left pulmonary arterial division, barium sulfate injected into the aorta could be seen in the pulmonary arteries as well as in the bronchial arteries (Fig. 2), indicating the presence of a precapillary anastomosis between the two systems. This was a constant finding in the left lung of animals that had undergone left pulmonary arterial division more than 2 weeks previously. In the right lung, on the contrary, filling of the pulmonary arterial tree with material injected into the aorta took place only when this lung showed evidences of having been involved

by a pneumonic process. Others[22-24] have shown that precapillary anastomoses between bronchial and pulmonary arterial systems develop in the presence of inflammatory conditions of the lung.

To determine the site of these anastomoses, neoprene and vinyl acetate casts were prepared and examined under the dissecting microscope. The neoprene casts proved more useful than the vinyl acetate casts in studying this problem because the specimens could be manipulated without fear of breakage. Anastomoses between bronchial and pulmonary arterial systems were encountered routinely in the periphery of the lobules just proximal to the smallest pulmonary arterial branches (Figs. 10 and 11). From the relation of these small vessels to the bronchial portion of the cast it appeared that the anastomoses were located just proximal to the smallest bronchioles prior to their entry into the alveolar ducts.

Occasionally, long fine vessels were seen in the hilar region running from enlarged bronchial arteries to the pulmonary artery. On closer examination, these vessels were found to run for short distances on the surface of the pulmonary artery and probably represented vasa vasorum.

Comment

According to Verloop,[12] the bronchial arteries of the rat arise commonly from the internal mammary artery. These vessels run along the bronchi as far as their smallest divisions. On microscopic examination the walls consist of a circular muscular layer and an internal elastic layer which is more prominent in vessels prior to their entry into the lung than within the lung. No precapillary anastomoses between the bronchial and the pulmonary arteries were demonstrated in Verloop's studies. According to Rakshit,[25] however, precapillary anastomoses occur in the hilar region in the normal rat.

The normal histologic characteristics of the rat's bronchial artery in the present study agree with those observed by Verloop.[12] Precapillary anastomoses between the two arterial systems were not encountered normally. The hilar precapillary anastomoses of Rakshit might be said to correspond to the occasional finding in this study of vasa vasorum of the pulmonary artery derived from the bronchial artery.

In studies conducted in the dog, Schlaepfer[2] noticed enlargement of the left bronchial arteries 66 days after left pulmonary arterial ligation. Mathes and her co-workers[3] noted moderate dilatation of the bronchial arteries of dogs 7 days after lobar pulmonary arterial ligation. There was further enlargement by 16 days. Liebow and co-workers[6] found an expanded bronchial collateral circulation in the dog 9 weeks after left pulmonary arterial ligation, which was well advanced by the

twelfth week. The studies here reported demonstrated marked dilatation of the bronchial arteries and expansion of collateral systemic vessels during the first 2 to 3 weeks after left pulmonary arterial division, with progressive changes of a lesser degree thereafter. The functional studies of Bloomer and co-workers[26] indicated that after ligation of the dog's left pulmonary artery, the greatest increase in systemic blood flow to the left lung occurred during the first few weeks after the operative procedure. These functional studies in the dog correspond to the anatomical findings in the present studies on rats. The rapidity with which collateral vessels could be demonstrated in the rats here described suggests that many such vessels may have developed from pre-existing vessels of capillary size, the latter dilating in response to stimuli set into action by ligation of the left pulmonary artery.

The microscopic changes reflected, in general, the sequence of events apparent grossly; that is, the greatest bronchial arterial dilatation seen under the microscope occurred during the first few weeks after pulmonary arterial division. Slight progressive changes occurred thereafter. Thickening of the walls of the bronchial arteries, although present in some specimens at 3 weeks, was not striking until 4 to 5 months after operation. With few exceptions this change was not as striking in the rat as in the dog.[6] The subendothelial fibrosis described in the dog by Liebow and co-workers[6] was not encountered in this study on the rat.

Demonstrable precapillary anastomoses between bronchial and pulmonary arteries developed rapidly, being present in some instances as early as 2 weeks after the beginning of the experiment. Other investigators have commented on the presence of anastomoses following interference with the pulmonary arterial circulation.[3-6,27,28] Mathes and co-workers,[3] studying dogs' lungs cleared by the Spalteholz method and specimens in which barium oxychloride had been injected into the aorta, encountered anastomoses between the two systems 25 days after ligation of a lobar pulmonary artery. These anastomoses were not numerous and occurred as short trunks connecting pulmonary vessels of moderate size with a bronchial arterial trunk, at a point where the two ran close together. They usually were encountered along pulmonary arterial branches of the third degree.

The rapidity of development of these anastomoses suggests their formation from pre-existing vessels of capillary size; and their location in the periphery of the lung in the region of the smallest bronchioles just proximal to the alveolar ducts speaks for their origin from communicating capillaries in the region of the respiratory bronchioles.

Capillary communications are known to exist normally in this location.[29,30] Such an interpretation was offered by Liebow and co-workers,[6] who demonstrated anastomoses between the two arterial systems, 50 μ and larger, occurring in the region of bronchi of the sixth order in dogs' lungs deprived of a pulmonary arterial circulation.

Summary and Conclusions

The bronchial arteries of the normal rat are small in comparison with the pulmonary artery and usually number no more than two or three for each bronchus. Histologically, the walls contain a circular muscular layer and an internal elastic layer. Precapillary anastomoses between the rat's pulmonary and bronchial arterial circulations are not normally demonstrable.

After division of the rat's left pulmonary artery, the left bronchial arteries dilate, become tortuous, and are apparently increased in number. At the same time collateral systemic vessels from other sources also supply the lung.

Increased thickness of the muscular layer develops and is associated with fragmentation of the internal elastic layer. The gross and microscopic changes occur rapidly within the first 2 to 4 weeks. Progressive changes continue to occur thereafter but at a slower rate.

Precapillary anastomoses between the left pulmonary and left bronchial arterial circulations could be demonstrated 2 weeks after left pulmonary arterial division. Neoprene casts of the rat's bronchovascular tree after ligation of the left pulmonary artery demonstrate these anastomoses in the periphery of the lung in the region of the smallest bronchioles proximal to the alveolar ducts.

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[*Illustrations follow*]

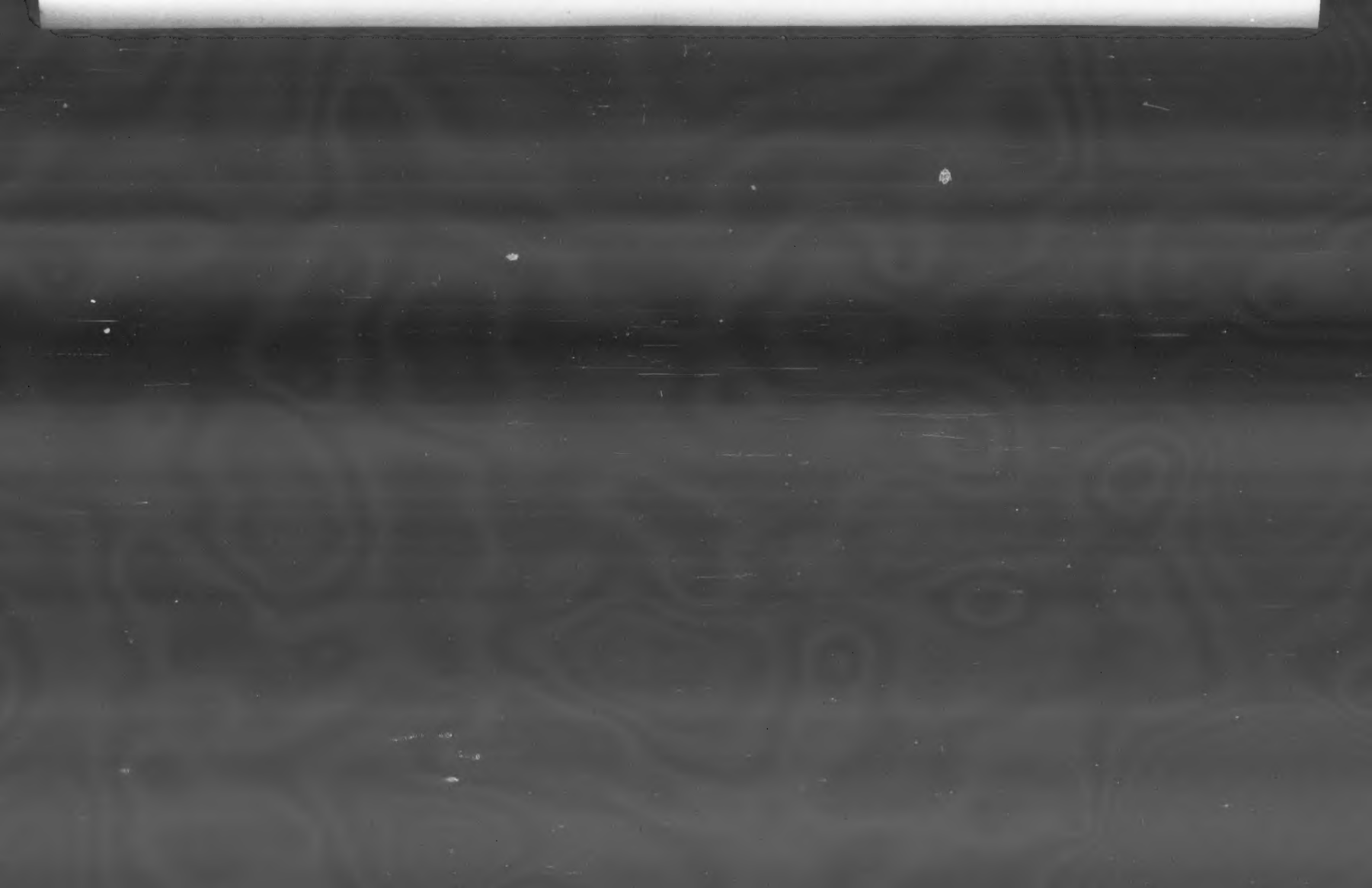
DESCRIPTION OF PLATES

PLATE 12

FIG. 1. Posterior view of a rat's lungs 3 weeks after division of the left pulmonary artery, showing increase in size, number, and tortuosity of the barium-filled bronchial arteries to the left lung.

FIG. 2. Rat's left lung 2 weeks after division of the left pulmonary artery. A small bronchial artery (B.A.) and pulmonary artery (P.A.) are both filled with a suspension of barium sulfate which had been injected into the aorta. Hematoxylin and eosin stain. × 190.

FIG. 3. Vinyl acetate casts of right and left bronchial arteries of a rat 6 months after division of the left pulmonary artery. The bronchial arteries of the left lung (right side of illustration) are enlarged, tortuous, and more numerous than those of the right lung (left side of illustration).



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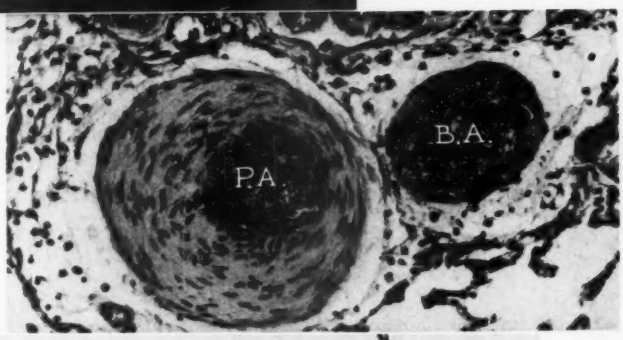

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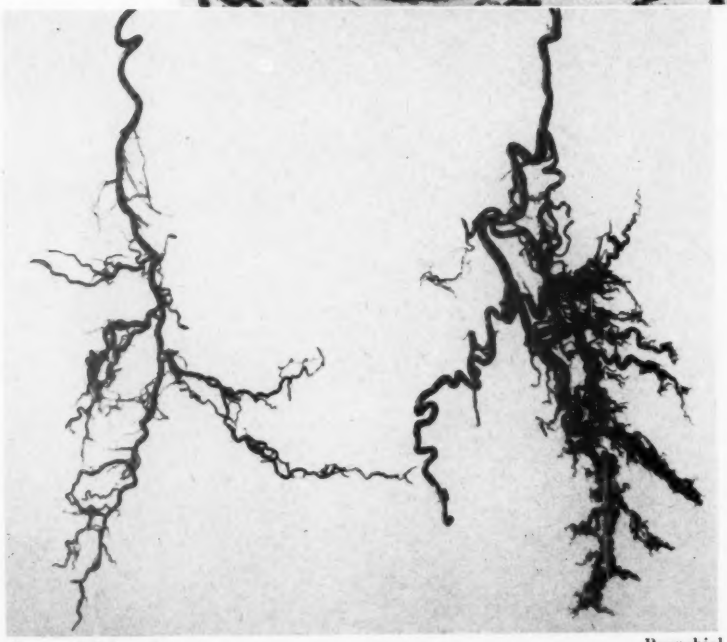

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PLATE 13

FIG. 4. Lobar bronchus of normal 210 gm. rat. B.A. = bronchial arteries filled with barium sulfate suspension. Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. × 85.

FIG. 5. Higher magnification of one of the bronchial arteries pictured in Figure 4. Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. × 580.

FIG. 6. Rat's left bronchus 3 weeks after division of the left pulmonary artery. Numerous dilated bronchial arteries filled with barium sulfate are seen in the wall of the bronchus. Hematoxylin and eosin stain. × 75.

FIG. 7. A small left bronchus of a rat 3 weeks after division of the left pulmonary artery. A dilated bronchial artery is encroaching on the bronchial lumen. Hematoxylin and eosin stain. × 250.

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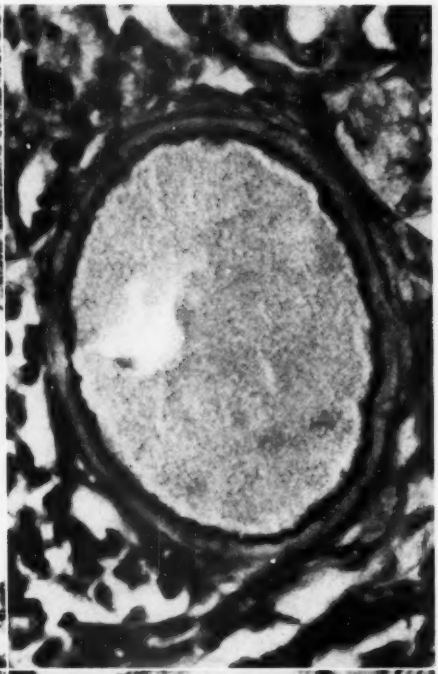

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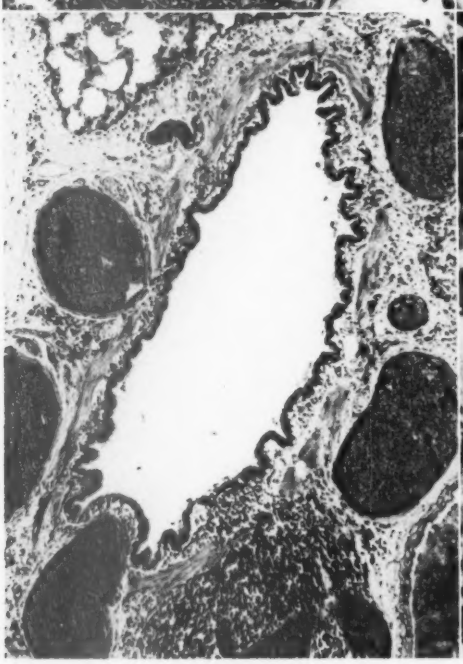

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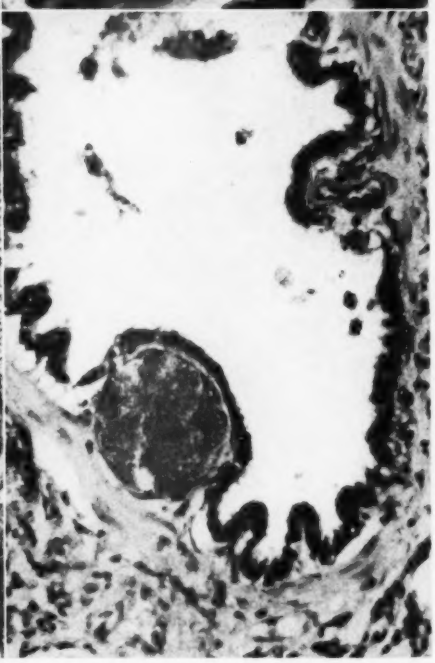


7

Ellis, Grindlay, and Edwards Bronchial Arteries

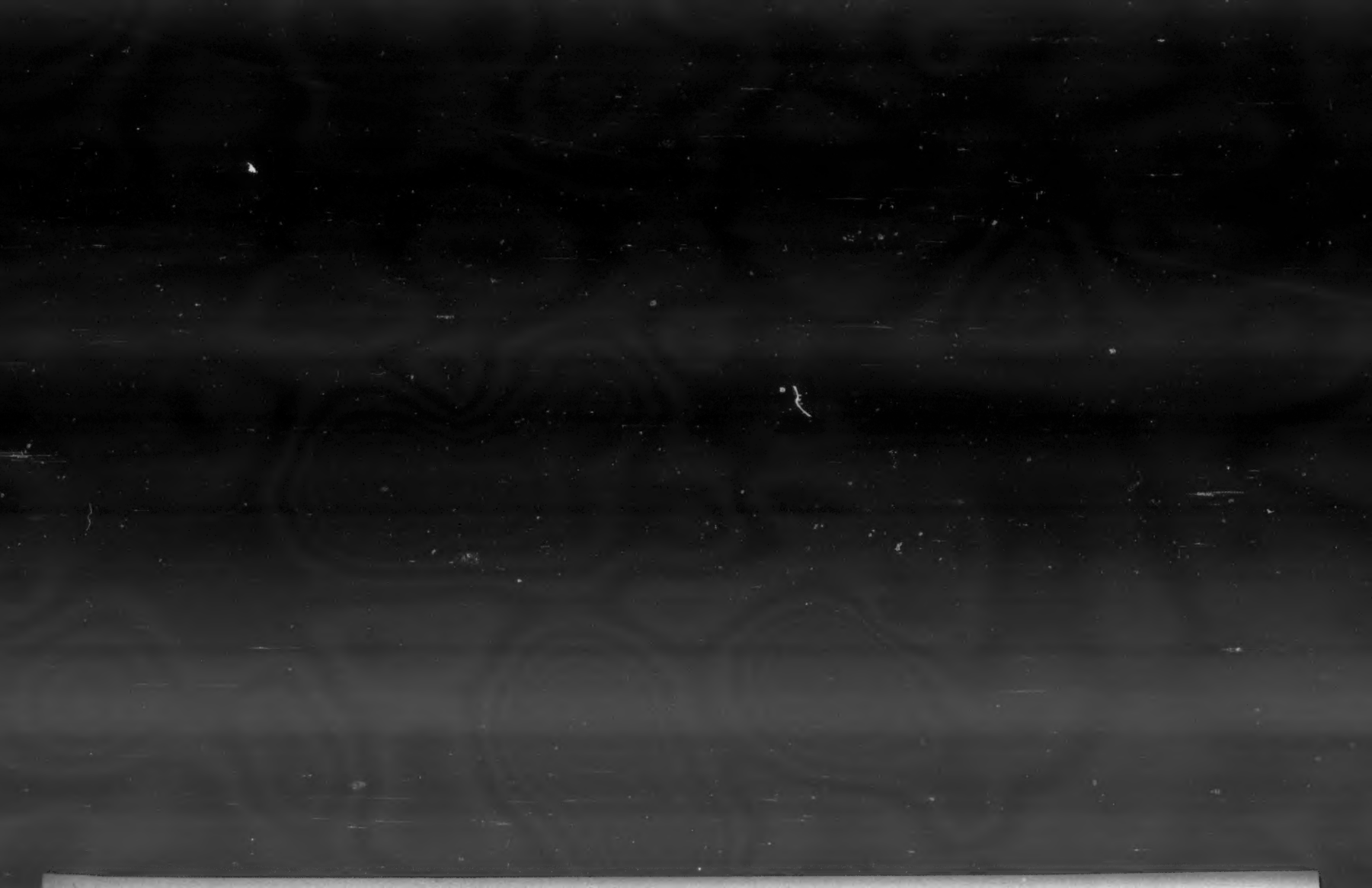
PLATE 14

FIG. 8. Rat's left bronchus 5 months after division of the left pulmonary artery. Four large, thick-walled bronchial arteries are visible. For comparison with normal (Fig. 4). Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. × 100.

FIG. 9. Higher magnification of a rat's left bronchial artery 5 months after division of the left pulmonary artery. The muscular media is thickened and the internal elastic membrane is fragmented in contrast to the normal (Fig. 5). Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. × 350.

FIG. 10. Neoprene cast of a rat's lung 5 months after division of the left pulmonary artery. × 18.

FIG. 11. Diagram of vessels illustrated in Figure 10.



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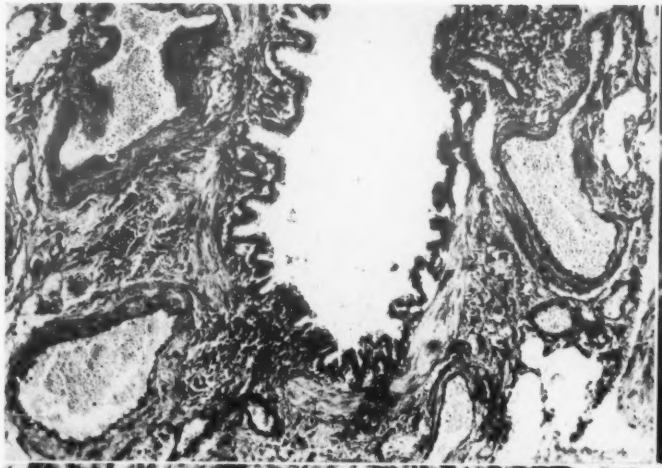

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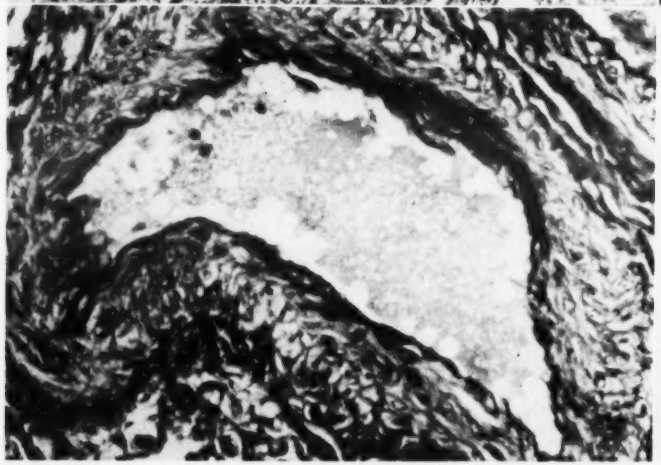

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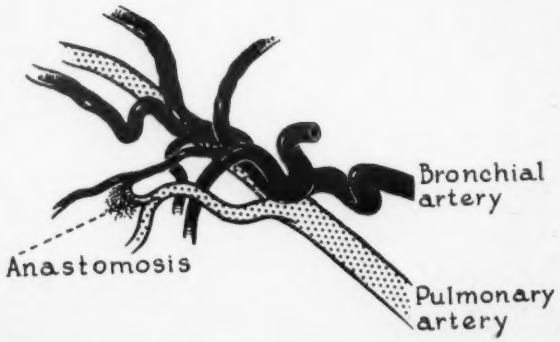

MORPHOLOGY OF THE MALIGNANT SQUAMOUS CELL

A Study of Six Thousand Cells Derived from Squamous Cell Carcinomas of the Uterine Cervix*

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The study of cellular structure has been employed extensively in investigating the female genital tract. As early as 1847, according to Papanicolaou,[1] Pouchet examined human vaginal smears, although this work now is of only historic significance. Our knowledge of the estrus cycle in animals is, in part, the result of cell studies made by many investigators, including Stockard and Papanicolaou,[2] Long and Evans,[3] Allen,[4] Selle,[5] Murphey,[6] and Corner.[7] Similar studies on the cellular elements in the human vaginal smear by Lehmann,[8] Papanicolaou,[9] King,[10] Ramírez,[11] and Moser[12] were concerned primarily with the physiologic process, although the use of the vaginal smear as a diagnostic aid was considered also. Thus, for at least 30 years, the study of exfoliated cells in vaginal smears has provided a useful experimental method for investigating the female genital tract, but only more recently has it been employed effectively as a means of detecting carcinoma.

A morphologic study of cells detached from tissues by physical means was used by early workers seeking a rapid method for microscopic diagnosis. Dudgeon and Patrick[13] reported on the use of wet-film preparations made by scraping the tissue with a scalpel. Employing a similar technic, Wrigley[14] studied lesions of the female genital tract and was able to identify uterine neoplasms readily by this means. Dudgeon and Barrett[15] further investigated the use of wet-film preparations in a detailed study and correctly detected 462 of 469 malignant tumors, including those of uterine origin. The interest in cytologic examination as a diagnostic procedure declined with the general acceptance of the frozen section technic.

During the course of his studies on the exfoliated cells in human vaginal smears, Papanicolaou[16] identified abnormal cells existing in the presence of carcinoma, and in 1943 Papanicolaou and Traut[17] published their monograph on the detection of uterine carcinoma by cytologic methods. Interest in the study of exfoliated cells was revived and the procedure was applied to other anatomical sites.[18] As a result,

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cytologic methods have provided a simple and useful approach to the early recognition of carcinoma.

The purpose of this investigation is to record in detail the morphologic features of neoplastic cells derived from squamous cell carcinoma involving the uterine cervix and to determine if possible the significance of these features in relation to the histopathologic findings.

Material and Methods

The specimens used in this investigation were chosen from over 20,000 cases accumulated by the Cytology Laboratory of this institution. The selection of specimens was based entirely on the abundance of malignant tumor cells present, although the ultimate distribution of cases was similar to that encountered in actual practice, with the less anaplastic neoplasms being more common. In many instances the cases utilized were those in which the cytologic interpretation was of material value in establishing the diagnosis.

The cytologic preparations were obtained either by scraping the uterine cervix or by aspirating the contents of the cervical canal. A tongue blade or the Ayre wooden spatula was used to scrape the cervical portio vaginalis and to spread the material over the surface of a glass slide. Cervical aspiration was obtained by means of a glass pipette and the secretion was expressed onto the surface of a glass slide. By applying a second glass slide and drawing the two apart, the apposing surfaces of both slides presented an even distribution of the material.

The tissue spreads were immediately immersed in a fixing solution composed of equal parts of 95 per cent alcohol and ether, and later stained by the technic described by Papanicolaou.[19] In general the EA36 modification was employed; however, the EA50* also was used in a few cases. Additional staining technics were utilized as will be indicated later in the discussion.

A minimum of 100 cells with morphologic features warranting classification as malignant tumor cells were studied in each of 60 cases so that in all over 6,000 cells were evaluated. All cases were examined by both investigators, each of whom studied 50 cells in order to check the validity of the findings and to assure a thorough coverage of the specimens. While this method of examination was used throughout the study, the findings in general were in close agreement despite the somewhat indefinite criteria which could be applied. The cellular features in each slide were recorded and measurements were obtained by means of an ocular micrometer and by the use of planimetry.

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In order to ascertain whether there was any correlation between the cytologic findings and the degree of anaplasia as determined by histopathologic examination, the tissue sections were reviewed and classified. Those cases of carcinoma *in situ* involving both surface epithelium and glandular spaces but without more definite evidence of invasion in the sections available were classified as group I, while group II included those cases in which there was, in addition, equivocal evidence of invasion. Group III included those cases in which there was invasive carcinoma arranged in well defined cell cords, while in group IV the process was more diffuse and cell cords were less prominent.

On histopathologic study the distribution of the cases was as follows: group I, 23 cases; group II, 8 cases; group III, 16 cases; and group IV, 13 cases. With the exception of the cases believed to be carcinoma *in situ*, the histopathologic classification was also a general index of the cellular differentiation of the neoplasm. Well differentiated tumors were common in group II, partially differentiated tumors in group III, and poorly differentiated carcinomas in group IV.

Cellular Morphology

Cellular Forms

A varied cellular configuration was encountered in this study. Of the 6,000 cells examined, 39.2 per cent were considered as being oval, 23 per cent were polyhedral, 20.2 per cent were more or less rounded, and 11.3 per cent were classified as irregular in outline. A total of 3.9 per cent were recognized as elongated forms, and only 0.7 per cent represented the so-called tadpole cells. There were 104 isolated nuclear forms surrounded by little or no discernible cytoplasm, accounting for 1.7 per cent of the cells examined.

All cases showed neoplastic cells which were classified as round (Fig. 1) or oval (Fig. 2). The highest incidence of either round or oval cells was seen in those tumors which were classified in group III on histopathologic examination. Polyhedral forms (Fig. 3) were more numerous in cells derived from carcinoma *in situ* and were less prominent in the more anaplastic neoplasms. Irregular cell forms (Fig. 4) were infrequent in the presence of neoplasms classified in group III and were more numerous in tumors classified in group IV. Thus the form of the neoplastic cells was of little significance, since varied configurations were observed in the cells from a given neoplasm.

The elongated cells (Figs. 7 and 8) identified in this study have been recognized by Papanicolaou and Traut[17] and other authors under a somewhat varied nomenclature, including streamer cells, fiber cells,

and pseudofibroblasts. The last is objectionable since neither the cell body nor the nuclear structure resembles that of the fibroblast. The cell form is characterized by an elongated cell body with well defined cytoplasmic boundary. While variable in size, it may attain several hundred microns in length, although smaller forms are more common. A total of 238 cells of this type were identified. They were more numerous in the presence of neoplasms classified in groups III or IV, and were common in group I as well. The highest incidence recorded in 100 cells was 15 per cent.

The term tadpole has been introduced by Papanicolaou and Traut[17] to describe an unusual cell form (Fig. 9) found in the presence of carcinoma. The cell body is characterized by a more or less rounded portion from which there extrudes a narrow, sometimes tapering, cytoplasmic process. Undue importance has been attached to this cell form which is uncommon in the presence of carcinoma, only 40 being identified in this series. The tadpole cells were more numerous in the presence of carcinomas classified as grade III and a maximum incidence of 7 per cent was found.

A more detailed subclassification of the cell forms seen in the presence of carcinoma seems unwarranted. The cellular pleomorphism so common in carcinoma studied by tissue sections is equally apparent in cell preparations, where the aberrant forms are frequently exaggerated. The method employed in making the tissue spreads contributes to the cellular pleomorphism and many cell bodies show varying degrees of elongation in a plane parallel to that of the spreading instrument. The distortion resulting in the tissue spreads is of some aid in the recognition of neoplastic cells, although it involves few cellular elements.

Cell Size

An exact measurement of size is difficult to establish when dealing with structures of irregular configuration, and cell area cannot be accurately computed from measurements of the greatest cellular dimensions. A more reliable determination of area can be obtained by the use of planimetry and the results of such a study will be reported at a later date.

The observations made in the course of this investigation do not permit detailed conclusions as to the size of the cell forms seen. In general the neoplastic cells varied only moderately in size. The variation encountered in those cells derived from more anaplastic carcinomas was not significantly different from that existing in the cells

from incipient carcinoma. This was not in agreement with our previous impression of a greater uniformity in the size of malignant squamous cells encountered in carcinoma *in situ*. The validity of these observations can be determined only by employing more accurate means of measurement.

The malignant tumor cells arising in neoplasms which had been irradiated showed a more marked variation in cell size, although such cases were few. This variation was not as marked as is observed in specimens from vaginal fluid.

Cell Membrane

The cytosome is limited by a membrane which can be resolved into an inner plasma membrane and an outer true membrane. The plasma membrane is usually not visible with the microscope but can be demonstrated by other means. It is concerned with cell permeability. A more resistant external membrane is well developed in plant cells where it serves a protective function. In animal cells, however, it is less prominent and more variable in nature.

A well defined peripheral limiting membrane was uncommon in the neoplastic cells of this series (Fig. 1). A well defined, regular cytoplasmic boundary accentuated in some cells by the suggestion of greater density in the peripheral cytoplasm was more commonly observed, with 55.9 per cent of the 6,000 cells studied showing this feature. An indefinite and less well defined cytoplasmic boundary (Fig. 4) was present in 42.4 per cent of the cells classified and in 1.7 per cent there were only isolated nuclear forms with little or no discernible cytoplasm (Fig. 4).

A poorly defined cell membrane has been considered by some authors as an important criterion for the recognition of the malignant tumor cell. Since the outer membrane is ill defined in many normal cells of the organism, the poor definition of this structure in many cells interpreted to be derived from carcinoma is not unusual.

The cells occurring in the presence of less anaplastic carcinoma as determined by histopathologic examination showed a somewhat higher incidence of well defined cell borders, and an equal predominance of ill defined cell boundaries was observed in cells derived from the more anaplastic carcinomas in group IV. This general statement as to the definition of the cytoplasmic boundary cannot be utilized to predict the degree of anaplasia present in the underlying neoplasm. The 100 cells studied from patients with carcinoma *in situ* frequently showed

well defined cytoplasmic borders; however, with similar histopathologic findings, the cell boundaries in fewer cases were consistently poorly defined.

The Cytoplasm

Of the 6,000 cells examined, 66.4 per cent had basophilic cytoplasm; in 30.9 per cent an acidophilia was observed; and in 2.7 per cent the staining reaction of the cytoplasm was considered to be indefinite. Neoplastic cells derived from the less anaplastic carcinomas showed a higher incidence of cytoplasmic basophilia, while 45 per cent of the cells showing an acidophilic cytoplasm were from neoplasms classified histopathologically as group IV.

Cytoplasmic vacuolization was noted in 2.2 per cent of the cells examined (Fig. 5). Of these, 69.6 per cent occurred in cells derived from carcinoma *in situ*. The vacuoles were either single or multiple and only rarely were they of such size as to displace the nucleus. The superficial location of carcinoma *in situ* and the poor nutrition in the cell masses probably account for the high incidence of degenerative changes such as vacuolization.

Cytoplasmic vacuolization was noted in 2.2 per cent of the cells examined and existed with equal frequency in the cells arising from tumors in each of the four histopathologic groups.

The cells identified in this study were more commonly isolated, and only 14.3 per cent were in 140 syncytial masses or tissue fragments. The latter were variable in size and were equally distributed throughout the four categories of neoplasm.

Concentrically arranged cell aggregates resembling epithelial pearls were identified in 5 instances and their distribution in the tumors was not significant. Such structures rarely occur in cellular preparations obtained from the uterine cervix and are of little significance unless their cellular constituents show definitely abnormal changes. The aggregate shown in Figure 6 was identified in the presence of squamous cell carcinoma but its cellular components are not unusual.

The Nucleus

Nuclear Forms

The configuration of the nucleus in the normal functioning and growing cells is related to the shape of the cytosome. Oval, round, and polyhedral cells have oval or round nuclear forms; and in cylindric or fusiform cells the nucleus is more elongated.

An oval nuclear form (Figs. 2, 12, 13, and 14) was present in 74.5 per cent of the cells in this series, while a round nucleus (Figs. 1, 16, and 17) was seen in 15.8 per cent of the malignant tumor cells. Irreg-

ular nuclear form (Fig. 10) was observed in 9.7 per cent of the cells. The incidence of oval and round shapes is more or less in proportion to the number of round, oval, or polyhedral cells identified. The nuclear forms with a more irregular outline, however, were not necessarily observed in cells classified as irregular but occurred in many different cell forms. The irregular nuclei included polypoid and lobate shapes and others characterized only by the term bizarre. A marked elongation of the nucleus was observed in the fiber cells of unusual length, and the nuclear outline in the so-called tadpole cell was in some instances a miniature replica of the cell shape.

Multiple nuclei (Fig. 15) were observed in 1.9 per cent of the cells examined and occurred with equal frequency in the cells arising from tumors in each of the four histopathologic groups.

Absolute evidence of mitotic division in the malignant tumor cells in this series was limited in extent. One cell was seen in metaphase (Fig. 11) and two additional cells were observed in anaphase. The location of two of these cells in syncytial masses offered proof of their epithelial origin, while the third mitotic figure was noted in a cell whose cytoplasm merged with that of an adjoining cell of neoplastic origin.

There were many additional cells whose nuclear patterns suggested an earlier phase of indirect cell division. In Figure 19 the nuclear structure is typical of that encountered in prophase. The nuclear membrane of this cell is ill defined and nucleoli are discernible. In many other malignant tumor cells the distribution of the chromonemata was similar to that observed in early prophase. In these cells, however, the nucleoli were not apparent. An impending mitotic division might easily explain the distribution of the nuclear chromatin in many of the cells encountered in this study.

*Nuclear Size**

The cells derived from normal stratified squamous epithelium show a marked variation in their nuclear size. The large polyhedral cells of superficial origin possess relatively small and sometimes pyknotic nuclei whose areas are frequently less than one-sixtieth of that of the cells, while smaller and more deeply lying cells in the parabasal zone are characterized by nuclei which occupy from one-third to one-fifth of the cell areas. This relationship between nuclear and cytoplasmic area can be expressed in the nuclear-cytoplasmic ratio which is useful in the recognition of the malignant tumor cell.

Many cells derived from the so-called atrophic cervical epithelium show nuclear-cytoplasmic ratios ranging from 1:2 to 1:5 as deter-

* Planimetry studies were conducted by Dr. D. G. Johnston. A more complete report on cytoplasmic-nuclear ratios in the cytologic diagnosis of cancer will appear elsewhere.

mined by planimetry. This is notable since many of the cells derived from carcinoma *in situ* show comparable ratios ranging from 1:2 to 1:4, although ratios greater than 1:2 also are encountered. In the presence of invasive carcinoma the neoplastic cells frequently show a nuclear-cytoplasmic ratio which is greater than 1:2 although many cells show ratios ranging from 1:2 to 1:3.

It is apparent that alterations in the nuclear-cytoplasmic ratio may be significant in some malignant tumor cells; however, there is a considerable variation. Isolated cells derived from apparently normal epithelium similarly may possess a markedly altered nuclear-cytoplasmic ratio; thus, even a marked change is not necessarily indicative of the neoplastic cell.

Nuclear Structures

The normal fixed and stained nucleus in the interphasic or metabolic period shows several basic component parts. These are: the nuclear membrane; the chromonemata, which contain the chromatin; chromocenters or karyosomes; the nucleolus; and the spaces occupied by the nuclear sap or karyolymph.[20]

The Nuclear Membrane

The nuclear membrane appears as a well defined structure, which according to Demerec[21] does not have the power of repair after rupture and collapses with the loss of karyolymph. It is observed in the interphasic nucleus and disappears during mitotic division.

The cells in this study were characterized by a nuclear membrane which was frequently accentuated (Fig. 14) and in some instances wrinkled (Figs. 3 and 18). The latter finding occurred in 20.9 per cent of the 6,000 cells examined and was observed in 71 per cent of the neoplastic cells derived from incipient carcinoma. It was less frequent in the cells arising from more anaplastic tumors. The frequency of this alteration of the nuclear membrane in cells derived from carcinoma *in situ* has previously been noted by one of us.[22] It is most likely the result of retrogressive changes in the cell and may be associated with intranuclear or intracytoplasmic vacuoles.

Chromonemata

The chromonemata appear as fine, irregular threads in the normal interphasic nucleus and contain a substance known as matrix; collectively these constitute the chromosomes.

A detailed study of the nuclear chromatin architecture apparent in the cells of this series resulted in a classification based on the pre-

dominant pattern encountered. The following categories were identified:

1. A uniformly finely granular pattern in which there were fine, distinct clumps of uniform size and staining reaction (Fig. 12).

2. A uniformly finely granular pattern with irregular clumps (Fig. 13) similar to the first noted basic pattern but with, in addition, larger aggregates scattered at random throughout the nucleus.

3. A uniformly finely granular pattern with strands (Fig. 14).

4. A uniformly finely granular pattern with irregular peripheral clumps (Fig. 14).

5. A uniform, but coarsely granular, pattern (Fig. 19).

6. An irregular coarsely granular pattern.

7. A translucent nuclear mass in which no definite granules, clumps, or strands were visible but which transmitted light.

8. An opaque, nuclear mass which did not transmit light (Fig. 10).

This somewhat general classification was employed in order to determine the most common nuclear pattern seen in the malignant squamous cell. A basic granular pattern was most commonly observed in this series. A uniform fine granularity with irregular clumping characterized the nucleus in 41.1 per cent of the cells studied, and a uniformly finely granular pattern was seen in 32.8 per cent of the cells. In 9.4 per cent of the cells the nucleus was classified as translucent and in 5.3 per cent an opaque nuclear mass was identified. The remaining nuclear patterns were less numerous and collectively represented 11.4 per cent of the 6,000 cells studied.

The relative incidence of the various nuclear patterns was the same in cells derived from tumors which were considered more anaplastic on histopathologic examination as in cells arising in less anaplastic lesions. A uniform distribution of each of the nuclear patterns was seen throughout the four groups established by examination of tissues and there was no significant statistical variation.

The varied nuclear architecture was in some instances investigated by cytochemical means. The most important nuclear component chemically is a nucleoprotein composed of simple proteins and nucleic acid, which is largely situated in the chromatin. The desoxyribonucleic acid content of the nucleus can be studied by means of the nuclear reaction of Feulgen, as shown by Feulgen and Rossenbeck[23] and later by Stowell.[24] When the Feulgen reaction was applied to the nuclei showing an opaque or translucent mass (Fig. 16), the desoxyribonucleic acid was apparent in granular or linear distribution (Fig. 17). The material accounting for the translucency or opacity was, perhaps, pro-

tein in nature. Quantitative measurement of the desoxyribonucleic acid in the nucleus in several cases revealed a significant increase in this component. This was true in the various nuclear patterns encountered. Further study by cytochemical means will be necessary to validate these observations, although Caspersson[25] has demonstrated that the nucleic acid content of the cell varies during mitosis, indicating that it plays an essential rôle in cell division.

It is these cytochemical features which are of importance in the production of the hyperchromatism which has been so long described as a feature of the malignant tumor cell. This, however, is not alone due to an actual increase in desoxyribonucleic acid but depends on the state of protein metabolism and other factors, study of which was beyond the limits of this investigation.

The Nucleolus and Karyosome

A well defined body, the nucleolus, is frequently present within the nucleus of the cytosome. Morphologically, in the fixed and stained preparation the nucleolus is sharply defined, usually rounded, homogeneous, and in general acidophilic. This is the true nucleolus, which histopathologically contains a ribonucleic acid, probably in the form of a nucleoprotein, according to Caspersson and Schultz.[26] Nucleolus-associated chromatin is frequently encountered. The latter obscures the presence of the underlying nucleolus and may rarely simulate the appearance of the so-called false nucleoli. These are aggregates of chromatin termed karyosomes or chromatin nucleoli which are similar histochemically to the chromatin filaments of the nucleus, being composed of histone, non-histone protein, and desoxyribonucleic acid, according to Mirsky and Pollister.[27]

Absolute histochemical identification of the true nucleolus is impossible without appropriate staining technics. However, a general idea as to the size and the incidence of this structure can be gained from the preparations in this series employing morphologic criteria alone.

In general, the cells interpreted to be derived from malignant neoplasms did not show prominent nucleoli when morphologic criteria were employed. While observed in a total of 16 cases, their incidence was significant in only 3 cases. Of 100 cells studied in each of these cases, 16, 89, and 95 revealed definite macronucleoli, while in the remaining 13 cases the greatest incidence was 3 in 100 cells. Thus the macronucleolus was a significant finding in only 3 cases and was unrecognized in the remaining 44 cases.

The macronucleoli were single or multiple (Fig. 4), and located in either central or eccentric positions. While usually rounded, they

were frequently irregular in outline and rarely presented bizarre forms similar to those noted by Hauptmann.[28] They measured approximately 4 to 5 μ in maximum dimension, thus justifying the designation macronucleoli. The exaggerated size of the structure, which far exceeds that seen in tissue sections, is probably due to distortion resulting from the method of preparing the tissue spreads.

Since some authors consider the nuclear-nucleolar area ratio of importance in the recognition of the neoplastic cell, these values were computed for the 3 cases showing more numerous macronucleoli. By means of an ocular micrometer the greatest and least dimensions of the nucleus and nucleolus were averaged and this value was assumed to be the diameter of a round mass of comparable size. A total of 300 cells were examined and in these the average nuclear-nucleolar area ratio was 25:1. Naidu,[29] in computing the nuclear-nucleolar area ratio of malignant tumor cells from lesions of the uterine cervix by more exact methods, arrived at a ratio of 26:1. The similarity in ratios suggests that the distortion produced by making the tissue spreads involves the nucleus and nucleolus to a comparable degree.

The large size of the nucleolus in many neoplastic cells has been noted by MacCarty[30] and other investigators as well. The infrequency of this finding in cells interpreted to be of neoplastic origin in this study deserves some comment. The staining technics employed were not ideal for detection of the nucleolus and yet in many cells the structure was clearly seen. The translucency or opacity of the nuclear mass could easily hide the nucleolus in some cells; however, other nuclei in which a finely granular chromatin pattern was observed similarly failed to show nucleoli. A marked increase in nucleolar size is apparent during intensive growth and occurs with cytoplasmic protein synthesis as stated by Caspersson.[25] Since many of the specimens in this series are from patients with histopathologic evidence of carcinoma *in situ*, the cells might be less likely to exhibit features common to cells of more malignant types.

The 3 cases showing numerous nucleoli are of interest since each specimen was obtained from a patient undergoing irradiation therapy. Although this has been noted previously in our material, it is by no means a constant finding after irradiation. Graham[31] has stated that the nucleolus is usually not apparent in neoplastic cells showing irradiation effect. There was only limited evidence of irradiation effect in either the neoplastic cells or non-neoplastic cells in these cases. In 2 cases there was clinical evidence of progression or recurrence.

The specimen showing the highest incidence of nucleoli was obtained from a patient with poorly differentiated squamous cell carcinoma.

However, there is insufficient evidence to permit any correlation between the frequency of nucleoli and the degree of anaplasia seen in the histopathologic material.

Summary and Conclusions

The more significant morphologic characteristics of the cells in this series are seen in the nucleus. These are by no means specific changes encountered in neoplastic cells alone, but are manifestations of altered cellular metabolism, of an unlimited growth potential, and in some cells of degeneration. The nuclear features in their relative order of importance are: structural changes in the chromonemata; altered nuclear-cytoplasmic ratio; hyperchromatism; variations in nuclear size and shape; and, when present, a macronucleolus.

The most common basic nuclear pattern encountered was a diffusely granular chromatin. This was in some instances associated with strands or with larger aggregates of variable size and shape. When the Feulgen technic was employed, these patterns and their variants were seen even in those cells which showed an opaque or translucent nuclear mass with routine staining procedures. The nuclear translucency or opacity cannot be explained on the existing cytochemical evidence. However, there is reason to believe that they represent retrogressive changes and may be the result of proteolytic activity.

A significant alteration in the nuclear-cytoplasmic ratio may be present in cells interpreted to be derived from carcinoma, but many cells derived from carcinoma *in situ* show a ratio within the range of that observed in atrophic parabasal cells during the menopause. In addition, isolated cells from an apparently normal cervical epithelium may show a nuclear-cytoplasmic ratio greater than 1:2. A high nuclear-cytoplasmic ratio is by no means common to all neoplastic cells.

Nuclear hyperchromatism was seen in many cells during the course of this study and is due to cytochemical changes. Quantitative changes in this respect may be difficult to evaluate and the relative intensity of the stain employed is variable. Drying of the cells frequently changes the staining reaction so that hyperchromia cannot be appreciated. The fundamental nuclear pattern is much more reliable in the recognition of the neoplastic cell than is the degree of hyperchromatism.

Tissue spreads commonly show a marked variation in the size and shape of nuclear forms in malignant tumor cells. However, this is not true of all carcinomas. This feature is perhaps overstressed in the literature and is not applicable to all neoplasms.

The macronucleolus can be a useful criterion when it exists. Many of the tumors in this series, however, did not show prominent nucleoli and their absence in cells obviously of neoplastic origin is not explained.

Cellular characteristics in addition to those pertaining to the nucleus are of significance in the recognition of malignant squamous cells but are somewhat less important. Abnormal cellular forms and variations in size and shape are seen; however, these are less common than nuclear changes.

The identification of malignant squamous cells in tissue spreads depends on a detailed study of the cellular components. Those specimens containing numerous cells with many of the features described are readily recognized as being derived from carcinoma; however, greater experience is required to evaluate specimens with limited changes in a few cells. A definite quantitative factor is involved in the interpretation of such specimens. This factor applies to both the changes encountered in the individual cells and to the number of cells present showing such changes. This can best be learned by experience. In general, a cell of undetermined type should possess at least two characteristics of the malignant tumor cell before being recognized as such. When these various cellular criteria are intelligently employed an accurate cytologic examination is possible. However, such interpretation should be based on the specimen in general rather than on an isolated cell.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 15

FIG. 1. An isolated spherical malignant tumor cell showing a well defined cell membrane. × 600.

FIG. 2. An isolated oval malignant tumor cell. × 600.

FIG. 3. A group of polyhedral cells derived from carcinoma *in situ*. The nuclei are variable in size and shape and one nucleus shows prominent wrinkling of the limiting membrane. × 600.

FIG. 4. A large irregular neoplastic cell characterized by an ill defined cytoplasmic boundary, alteration in the nuclear-cytoplasmic ratio, granular chromatin, and prominent nucleoli. Small cell forms also are seen, several of which show little or no cytoplasm. × 600.

FIG. 5. Cells derived from carcinoma *in situ* showing variation in nuclear size and shape. The nuclear membranes show limited wrinkling and nuclear degeneration is evident. Cytoplasmic vacuolization is present in several cells. × 600.

FIG. 6. An epithelial pearl whose component cells are not sufficiently altered to warrant classification as neoplastic cells. × 600.

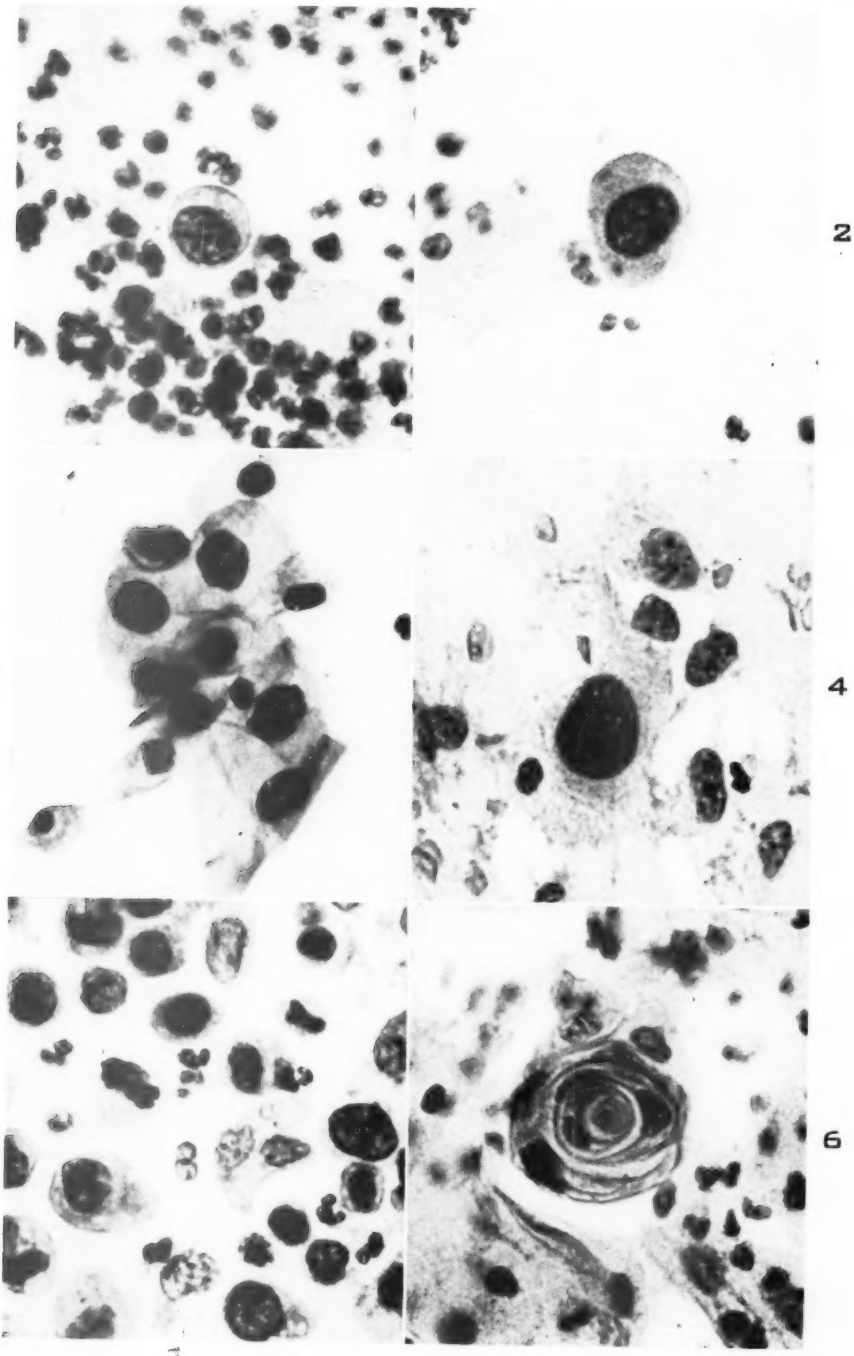
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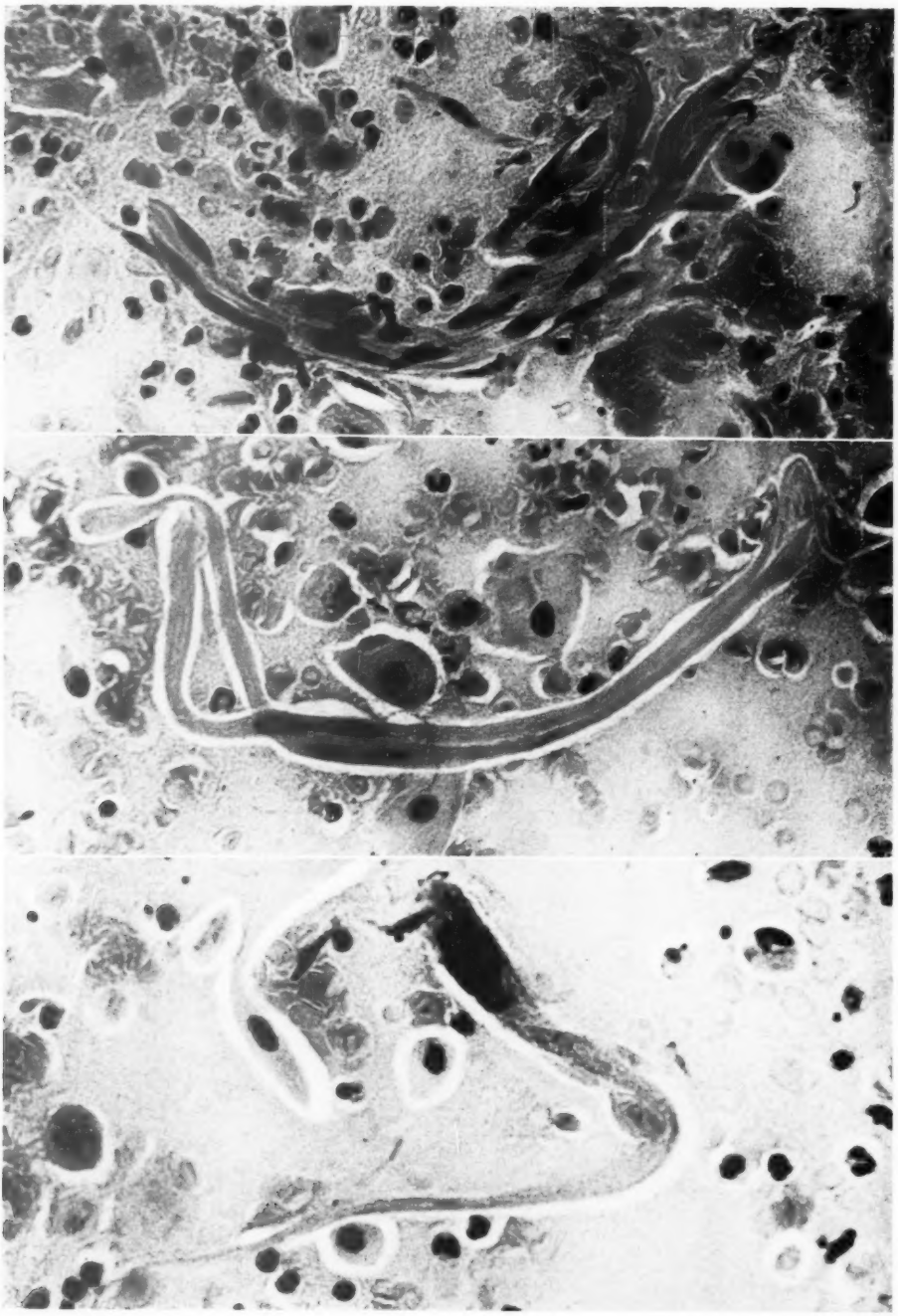
PLATE 16

FIG. 7. A group of elongated cells derived from squamous cell carcinoma. × 420.

FIG. 8. Unusually elongated cells. × 420.

FIG. 9. An example of tadpole cell. × 420.



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Reagan and Moore The Malignant Squamous Cell

PLATE 17

FIG. 10. Cells derived from carcinoma *in situ* showing variation in nuclear size and shape. There is limited wrinkling of the nuclear membranes. Two cells show irregular and opaque nuclear forms. × 600.

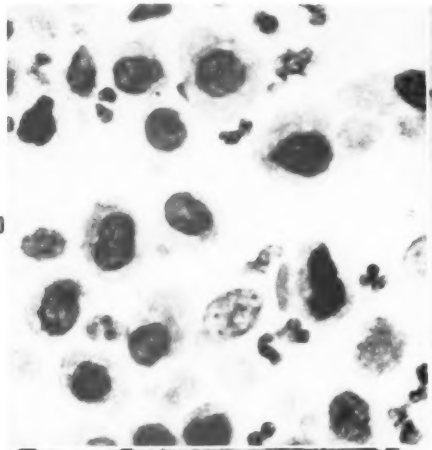
FIG. 11. A cell in mitotic division (metaphase). The cytoplasm merges with that of the contiguous malignant tumor cell. × 600.

FIG. 12. The nuclei in these cells show a more or less uniform, finely granular pattern. × 600.

FIG. 13. An isolated cell whose nuclear pattern is considered finely granular with irregular clumping. × 600.

FIG. 14. Nuclei of variable size show a basic, finely granular chromatin pattern. The chromatin is also in strands and in isolated aggregates of larger size. One nucleus shows limited peripheral clumping of chromatin. × 600.

FIG. 15. Binucleate and trinucleate malignant tumor cells. × 600.



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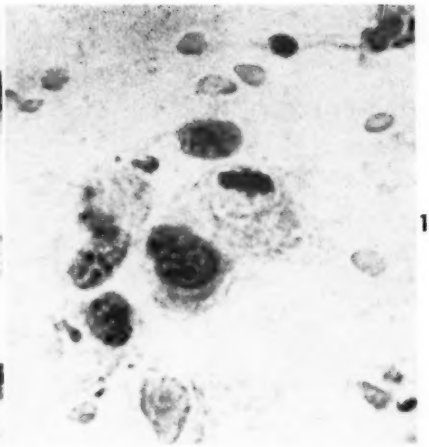

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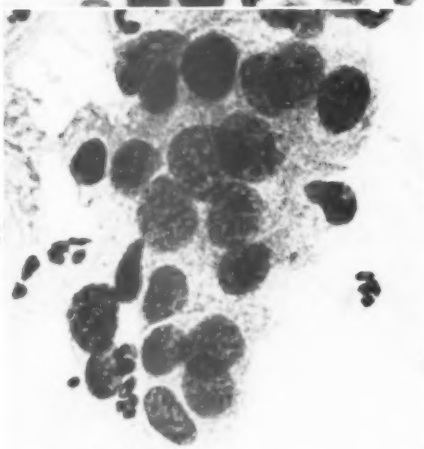

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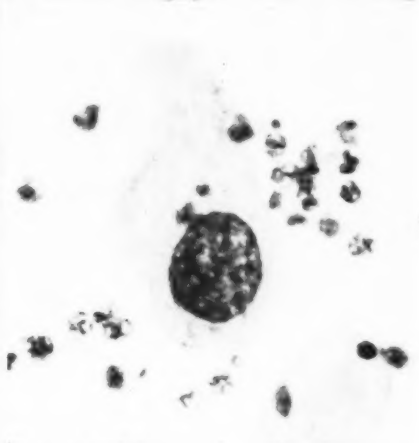

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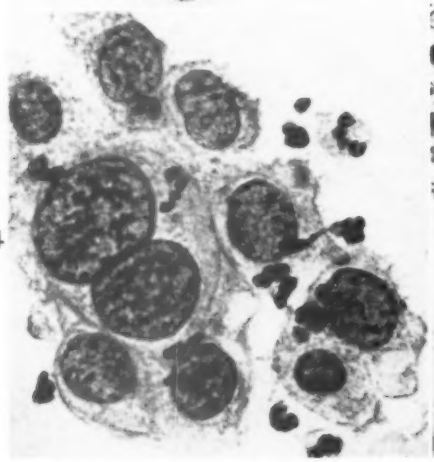

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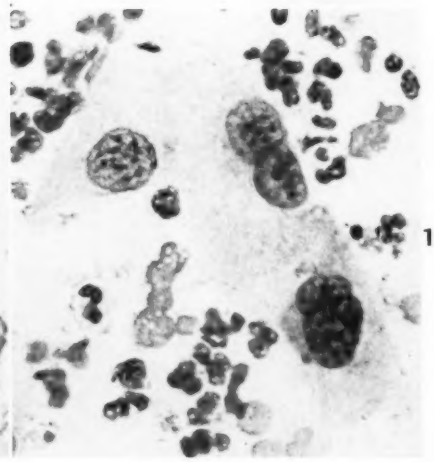

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PLATE 18

FIG. 16. Two malignant tumor cells whose nuclei are somewhat translucent as stained with EA36. × 600.

FIG. 17. The cells shown in Figure 16 when stained with the Feulgen reaction to show the disposition of desoxyribonucleic acid. × 600.

FIG. 18. A group of cells showing marked wrinkling of the nuclear membranes. × 600.

FIG. 19. An isolated cell showing a nuclear pattern considered to be uniformly and coarsely granular. The disposition of the nuclear chromatin, the poorly defined nuclear membrane, and discernible nucleoli are suggestive of a prophase stage of mitosis. × 600.

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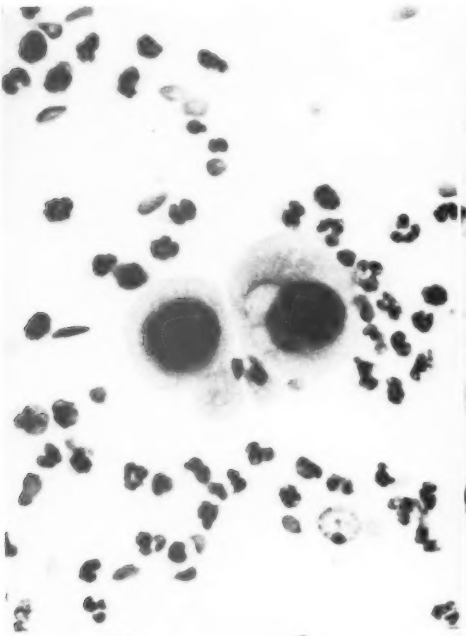

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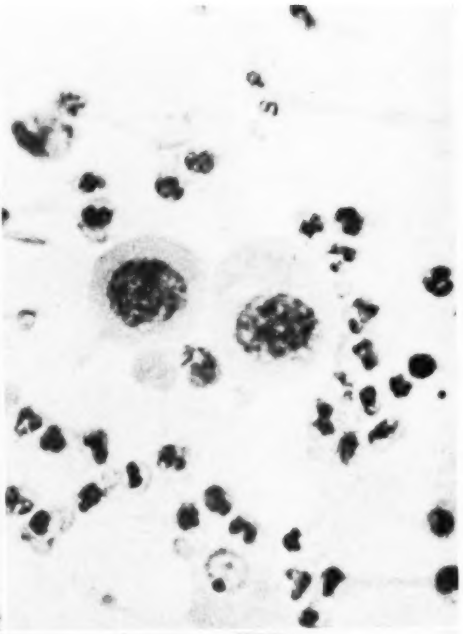

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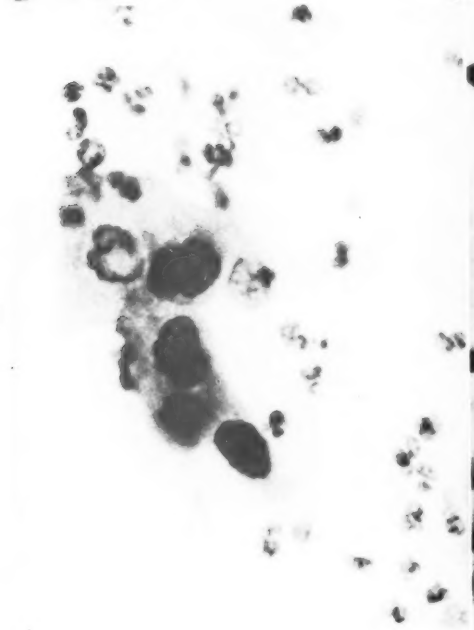

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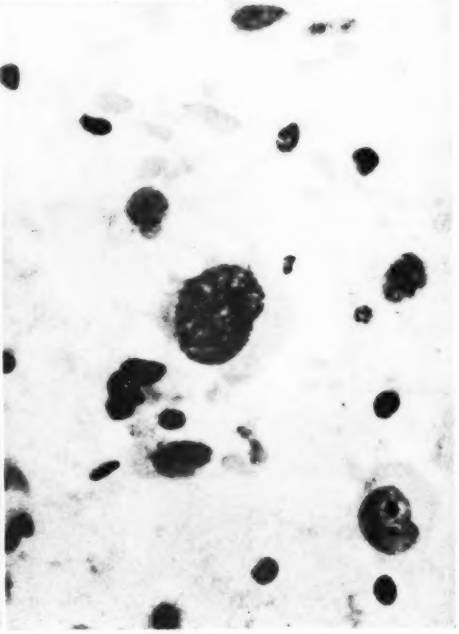

Reagan and Moore The Malignant Squamous Cell

MORPHOLOGY OF THROMBOTIC THROMBOCYTOPENIC PURPURA WITH DEMONSTRATION OF ANEURYSMS*

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The disease entity that has been called thrombotic thrombocytopenic purpura[1] and thrombocytic acroangiothrombosis[2] is characterized clinically by thrombocytopenic purpura, hemolytic anemia, and varied neurologic manifestations. The outstanding histologic lesion is occlusion of the small arteries, arterioles, and capillaries by amorphous or granular acidophilic material. These occlusions were first reported by Moschcowitz[3] in 1925 using the descriptive term hyaline thrombi. In 1936, Baehr, Klemperer, and Schifrin[4] published the next report of the disease and, in addition to correlating the clinical and pathologic manifestations, postulated that the occlusions are composed of platelets. These lesions have been described in almost every organ in the body but have been seen only rarely in the lung. All observers since Baehr *et al.* have accepted the hypothesis that these occlusions are composed, at least in large part, of platelets.

Altschule,[5] in 1942, first suggested that the occlusions might be secondary to a primary vascular change. Several investigators[6-9] have since pointed out lesions of the vessels associated with the occlusions. In a recent study Gore[10] has demonstrated a "prethrombotic" vascular lesion and has concluded that this is the initiating factor in the occlusion.

A prominent and apparently characteristic and significant lesion of this disease is the marked vascular dilatation frequently associated with the occlusion. That this takes the form of an actual aneurysm has not been reported previously, although several investigators[4,7,10,11] have mentioned the dilatation or distention of the vessels. Since these aneurysms are such a prominent manifestation, they form the basis for this report.

Material and Methods

A clinical-pathologic report of the 2 cases from which this material was taken has been made by Meacham, Orbison, Heinle, Steele, and Schaefer.[12] The majority of this material came from their rapidly fatal case 1. Histologic examination was made of sections from all of the organs of these cases and special stains were used on selected tissues to demonstrate more clearly certain features of the vascular

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lesions. These included the elastic tissue-van Gieson, trichrome, and phosphotungstic acid-hematoxylin stains, and the Feulgen, and periodic acid-leukofuchsin reactions.

In order to obtain a three dimensional concept of the aneurysms, it was decided to reconstruct involved vessels from serial sections. A block was selected at random from the wall of the right ventricle of the heart of case 1 and sectioned serially at a thickness of 10 μ. The sections were mounted in sequence and stained with hematoxylin and eosin. A total of 180 sections were examined. By using the scopicon* as a camera lucida, drawings of each section were made on cardboard at a magnification of 260 $\times$. The magnification was determined by dividing the actual diameters of several structures into the diameter of their reproductions. The actual diameter of microscopic structures was determined by the use of a calibrated ocular micrometer. The cardboard (chipboard 0.025) measured 1/40 of an inch or 0.62 mm. thick, so that four thicknesses represented one section and measured 2.5 mm. thick instead of the calculated 2.6 mm. By cutting out the cardboard drawings in quadruplicate and gluing them together, a rough model was constructed, representing 143 consecutive sections. It was not possible to put the whole model together without its becoming so unstable that it collapsed. Therefore, it was left in sections and a final reproduction was made from it in clay by free-hand modeling on a wire framework. The wire used was an aluminum armature wire obtainable at artists' supply shops and the clay was an amateur-grade modeling clay. By this method, a model was obtained that was stable. Details that were not clearly defined in the scopicon image were added at this time by direct microscopic examination of the sections.

Observations

The presence of amorphous and granular occlusions in the arterioles and capillaries with varying degrees of proliferation of endothelial cells was the most striking change in these cases. In agreement with Gore's[10] experience, it was found that both platelets and this occluding material are eosinophilic and stain positively with the periodic acid-leukofuchsin reaction. Inflammatory cellular exudate was not seen with these lesions, although hemorrhage was occasionally observed.

The walls of the arterioles frequently contained a characteristic lesion in which a homogeneous, acidophilic mass (fibrinoid) replaced the usual cellular and fibrillar structure, including the elastic lamina. This was a smudgy lesion involving the total thickness of the wall and

* Scopicon, Inc., 215 E. 149 St., New York 51, N.Y.

occasionally the adjacent connective tissue, but extending through only a small segment of the total circumference. Such lesions occasionally were seen without any intraluminal occlusion (Figs. 1 and 2). When they were associated with intraluminal masses, the material in the lumen frequently was attached to and continuous with the material in the wall (Figs. 3 and 4). These lesions occurred also in arterioles that were greatly dilated, but then it was often necessary to identify the focal lesion by its homogeneous, acidophilic character alone, since attenuation of the vessel wall with extensive loss of the elastic lamina rendered the elastic stain of little value.

In both cases and especially in the acute one, dilatation of the involved vessels was a striking feature. These aneurysms were identified in brain, thyroid gland, liver, adrenal gland, kidney, stomach, mesentery, and especially the myocardium. By studying the reconstruction of a portion of the arterial supply of the right ventricle (Figs. 8 and 11) it was seen that many small arteries, arterioles, and capillaries showed aneurysms. The aneurysms in the small arteries and arterioles were for the most part of cylindric type and, although long, usually were only moderately dilated. One globular aneurysm was demonstrated in a small artery (Fig. 11). The site most markedly and consistently involved by aneurysms was the region of the arteriolar-capillary junction (Figs. 8 and 11). The reconstruction revealed nearly every identified junction to be involved. Many of the aneurysms at these sites were similar, being roughly fusiform (Figs. 7 and 10). This form was distorted, however, when more than one capillary arose from the same aneurysm.

The aneurysms in the small arteries and arterioles contained amorphous acidophilic material covered by a single layer of endothelium and usually attached to the wall at one or more points (Figs. 3 and 12). At these points of attachment, the elastic lamina frequently was absent, and the media and adventitia showed varying degrees of homogenization and acidophilia, frequently marked. The remainder of the wall often was attenuated, with loss of its elastic membrane; but occasional aneurysms had only focal destruction of elastica. At the arteriolar-capillary junctions, the walls of the aneurysms were composed entirely of a single layer of endothelium and contained varying proportions of the amorphous-acidophilic material and endothelial proliferation. The endothelium not only covered the amorphous masses but also formed uninterrupted expanses of cells. In some instances, endothelial proliferation occurred without associated "thrombus" (Fig. 9). One aneurysm (Fig. 15) had an amorphous acidophilic wall

(fibrinoid degeneration), and was the only one associated with extravasated blood. No alterations of veins were identified during the reconstruction.

Discussion

The most notable histologic feature of this disease and the one which all writers have discussed is the vascular occlusion. Since Baehr *et al.*[4] first postulated that the occlusions are platelet thrombi, all investigators have agreed that such a composition is most likely, and some have stated the belief without qualification. Since no specific test for platelets in tissues is available, the conclusion has been based on the structure of the lesions,[4] the demonstrated absence of red cells, white cells, and fibrin,[2,4] and the fact that the occlusive material and centrifuged platelets give similar staining reactions.[10] However, none of these tests is specific and therefore they do not justify an unqualified conclusion.

Altschule[5] suggested that a lesion of the endothelium is possibly the primary event in this disease and is followed by the formation of platelet thrombi. Although Green and Rosenthal[11] and Bernheim[13] found endothelial proliferation only in association with thrombi and thus considered the endothelial reaction secondary to thrombosis, Trobaugh *et al.*,[6] Engel *et al.*,[7] Carter,[8] and Muirhead *et al.*[9] all reported endothelial proliferation in the absence of thrombi and supported the possibility of a primary vascular lesion. In addition, Engel *et al.* and Carter reported degenerative and destructive changes in the walls of the vessels. Gore[10] demonstrated "prethrombotic" lesions of hyaline material in the intima of the vessels and considered this the primary lesion upon which platelet thrombi developed. The observations on the 2 cases reported here support the belief that a lesion of the vessel wall does occur and that it is probably primary.

Two investigators[6,13] have mentioned the involvement of veins or the presence of perivenous hemorrhage. I have not observed similar changes, and it seems probable that the venous involvement and perivenous hemorrhage previously described have actually been thin-walled arteriolar aneurysms that have been understandably mistaken for veins when studied in single sections. In many instances, accurate identification is possible only by following the vessel through several sections of a series.

The remarkable aneurysmal distortion that occurs in the distal portion of the arterial tree and in the capillaries in this disease is shown in Figure 8. The previous lack of study of this feature is probably explained by the fact that quantitative alterations in the diameter of a sectioned vessel are less impressive than the qualitative alteration

of occlusion. And yet, if the occlusions occurred only in vessels of normal dimensions, the histologic appearance would be much less conspicuous than is the case. That such aneurysmal dilatation is the result of platelet thromi alone seems very unlikely. The usual course of events in aneurysmal formation is a destructive lesion of the wall of the vessel followed by dilatation and often by thrombosis. Such a course of events is suggested in the present instances by the fact that not only are the walls of the aneurysms attenuated, but they frequently have lost their elastic laminae. Further support of such a hypothesis is supplied by the fact that degenerative and destructive lesions also are seen in arterioles in which aneurysmal formation has not occurred. Thus, it is believed that the development of aneurysms in this disease is significant additional evidence for a primary vascular lesion.

The evidence now available supports the conclusion that the histologic changes in this disease are manifestations of an injury to the walls of small arteries, arterioles, and capillaries and that the dilatations and occlusions are secondary. The reported vascular lesions include: (1) the "prethrombotic" hyaline changes[10]; (2) the focally destructive lesion of the vessel wall described in this report; (3) the extensively destructive lesions frequently associated with occlusions of the vessels[7,8]; (4) the endothelial proliferation in the absence of occlusions[6,8,9]; and finally (5) the aneurysmal alterations studied for this report. To conclude that all of these changes are secondary to agglutinated platelets requires that platelets infiltrate and destroy the structures of the vessel wall and produce marked dilatation of the vessel. Such a mechanism is not supported by any work known to us, whereas the conclusion that the lesions in the vessel wall are primary and result in occlusions and dilatations of the vessels is in accord with previous experience in vascular disease.

There is not yet sufficient evidence to allow us an unqualified conclusion concerning the composition of the occlusive masses. Whether they are composed of platelets as commonly accepted, a combination of platelets and degenerated material as suggested by Gore,[10] or a protrusion of degenerated material into the lumen is not finally determined. It has been pointed out[10] that centrifuged platelets and the occlusive material in the vessels in this disease respond similarly to several stains. However, none of these stains is specific for platelets and the most that can be concluded is that platelets and the occlusions contain some similar reactive groups. As a matter of fact, the intramural lesions of this disease react in exactly the same way as the platelets and the occlusive material when exposed to the same stains. Since it is very unlikely that the intramural lesion is one of platelets, Gore

has suggested that the lesion may be a bimorphic one in which the base is degenerated material of the vessel wall and the superficial part is composed of platelets. Further elucidation of this problem must await specific or differential cytochemical technics.

As might be anticipated, it has been suggested repeatedly that this disease is related to that group called the collagen diseases. Such a relationship seems reasonable, but I consider this to be a disease entity within the group of collagen diseases rather than a variant of one of the previously characterized diseases. This stand is supported by the prominent aneurysms, large occlusive masses in arteries and capillaries, and the focal destructive lesions of the arteries which characterize this disease and are not found in the other members of the group.

In speculating on the possible pathogenesis of this disease, the work of two investigators may be cited. Bedson[14] has shown that thrombocytopenia alone will not produce purpura and that some other factor, presumably injuring the vessel wall, is required. He also stated that in purpuric animals the presence of swollen endothelial cells supports the conclusion of vascular injury. Humble,[15] by direct microscopy of the vessels of the skin in purpuric patients, has reported that all of the bleeding occurs at the arteriolar-capillary junctions. Since I find the aneurysms in this purpuric disease predominantly at the arteriolar-capillary junction and since there is good evidence of vascular injury, it may be postulated that in this disease the causal agent is different from that in ordinary thrombocytopenic purpura in that it produces an injury which results not only in thrombocytopenia but also in hemolytic anemia and vascular degeneration of such a nature that aneurysms and vascular occlusions occur.

Summary

A histologic study of 2 cases of thrombotic thrombocytopenic purpura corroborates previous reports that amorphous eosinophilic occlusions of the small arteries and arterioles are a prominent feature of this disease. Preliminary attempts to identify the nature and origin of these occlusive masses by cytochemical technics failed.

Examination of the arterial walls revealed focal amorphous eosinophilic lesions which replaced the normal components of the wall and in some instances were continuous with the intraluminal masses. These observations support the concept of a primary rather than a secondary arterial lesion in this disease.

A cardboard reconstruction (260 ×), later remodeled in clay, was made of a portion of an artery and its branches from serial sections of a block of the right ventricular wall. The reconstruction demonstrated

cylindric and occasional globular aneurysms of the small arteries and numerous large fusiform aneurysms at the arteriolar-capillary junctions. These findings also support the concept of a primary arterial lesion.

No involvement of veins or venules was found by this study of serial sections.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

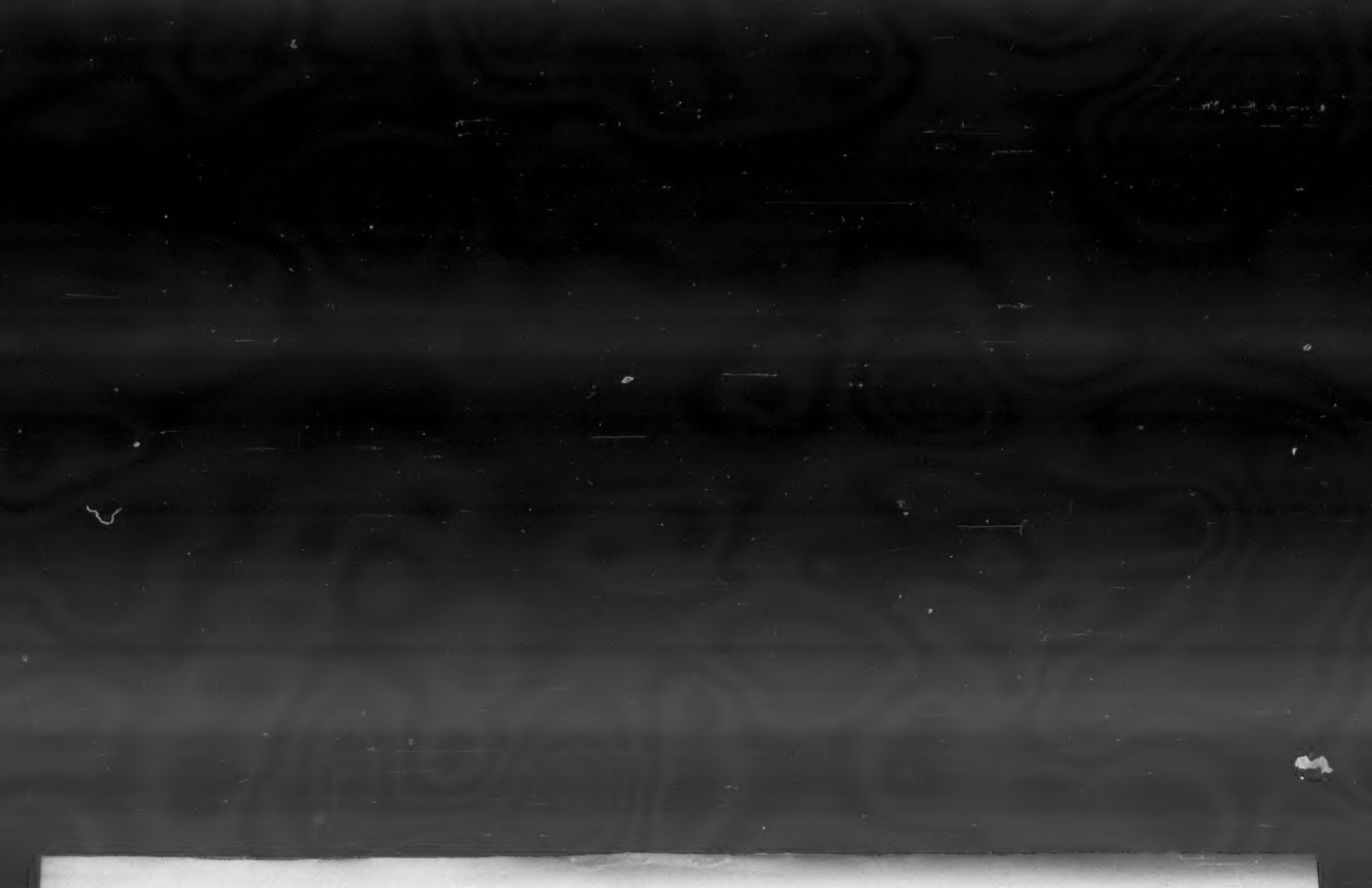
PLATE 19

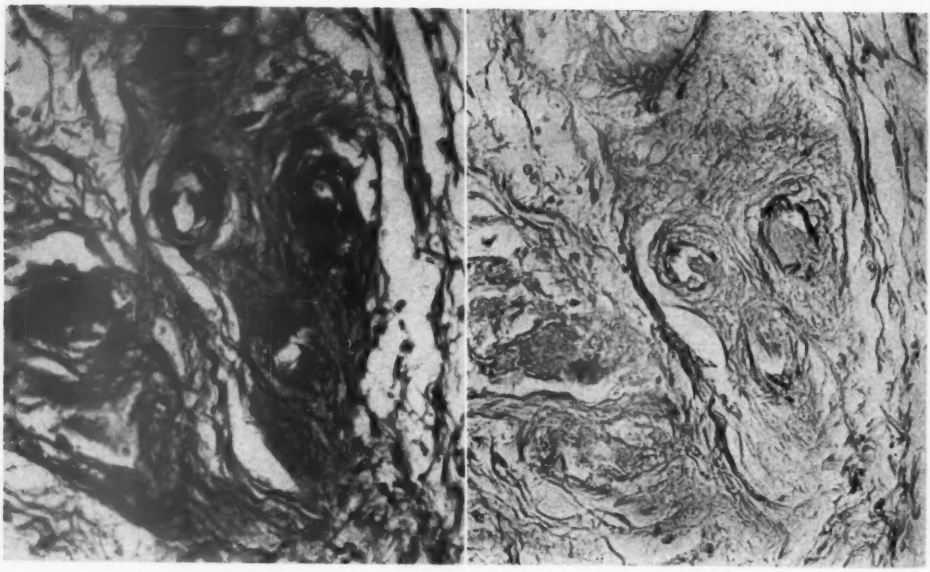
FIG. 1. Photomicrograph of three arteries in the epicardium stained by the periodic acid-leukofuchsin technic to demonstrate foci of intramural degeneration (black smudgy foci). × 286.

FIG. 2. Photomicrograph of section adjacent to that in Figure 1, stained by the elastic tissue-van Gieson technic to demonstrate the focal loss of elastic tissue in the intramural lesion. × 286.

FIG. 3. Photomicrograph of a small artery in the myocardium stained with hematoxylin and eosin to demonstrate the partial occlusion and fusion with the vessel wall. × 286.

FIG. 4. Photomicrograph of section adjacent to that of Figure 3, stained with elastic tissue-van Gieson technic to demonstrate the destruction of the elastic tissue at the site of continuity between intraluminal material and the vessel wall. × 286.





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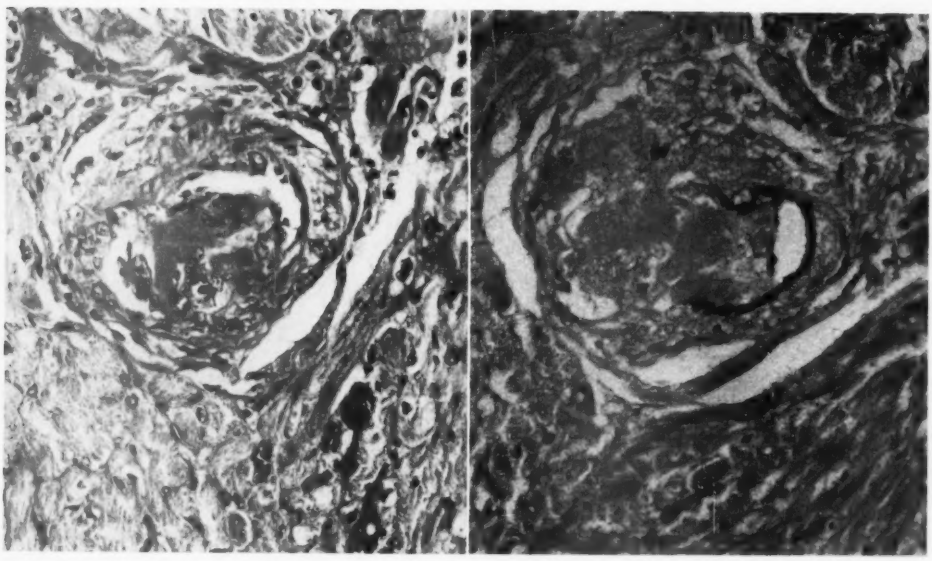

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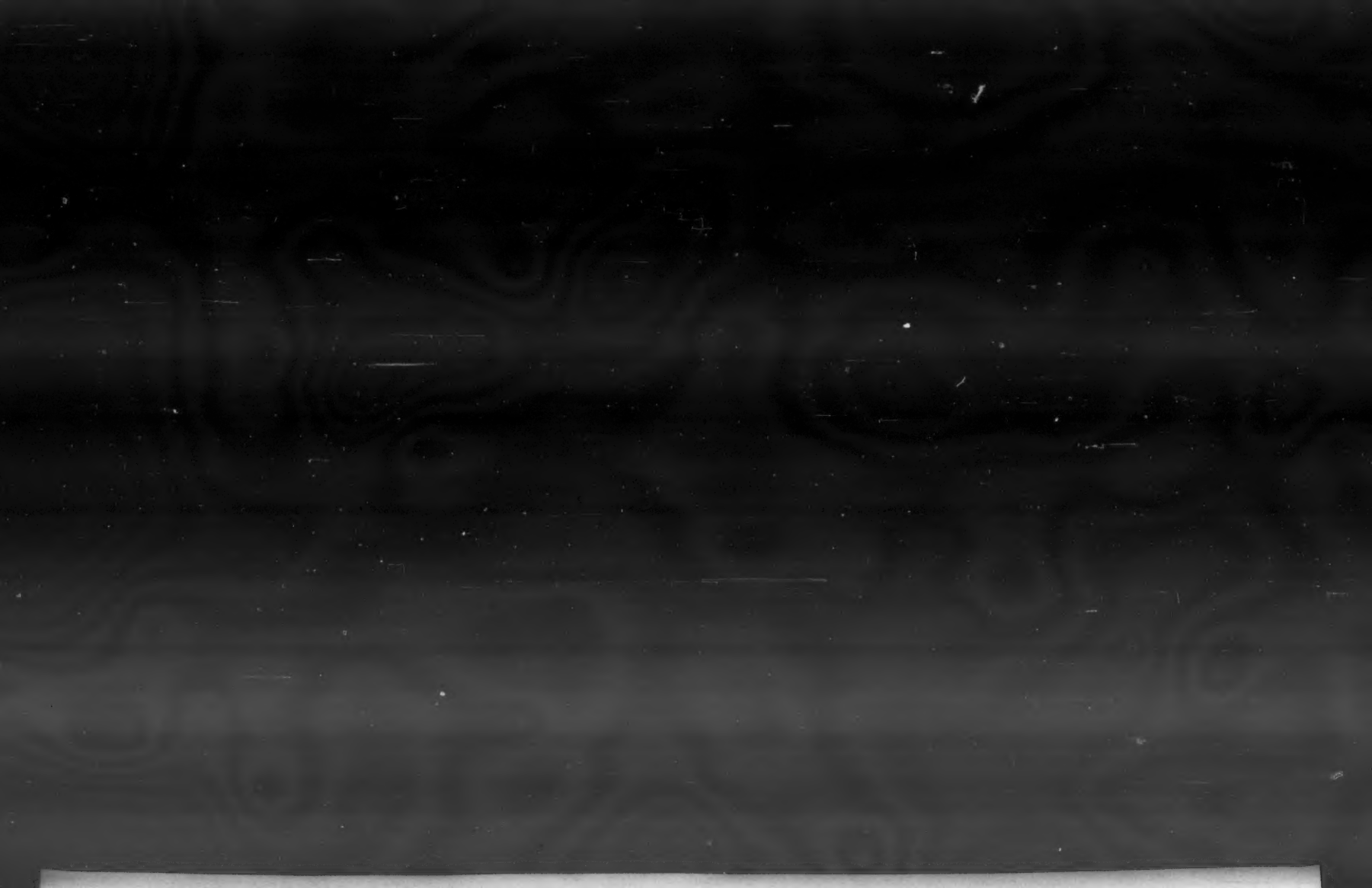
Orbison Thrombotic Thrombocytopenic Purpura

PLATE 20

FIG. 5. Photograph of a portion of the original cardboard model. The aneurysms arising from the small vertical arteriole at the left have dropped together because of the instability of the model, thus obscuring their outlines.

FIG. 6. Photograph of a portion of the final clay model illustrating the region shown in Figure 5. By this technic individual structures are readily identified and the model is stable.

FIG. 7. Photomicrograph of a section taken at the site of the black line in Figure 6. The arteriole on the right opens into a large aneurysm at the arteriolar-capillary junction.



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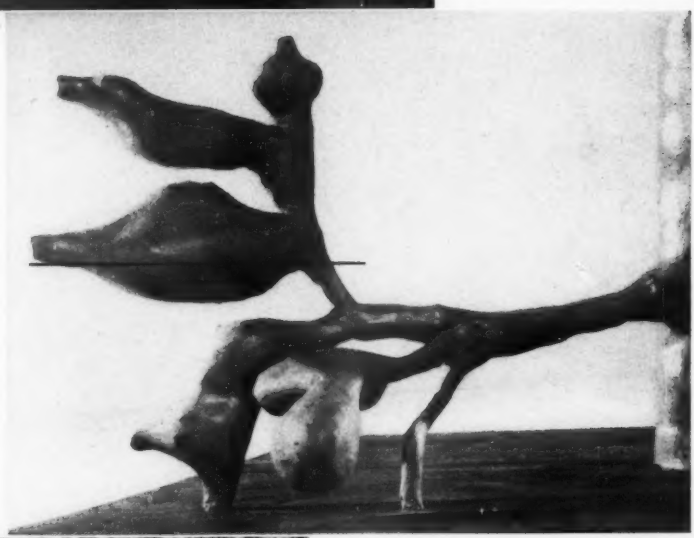

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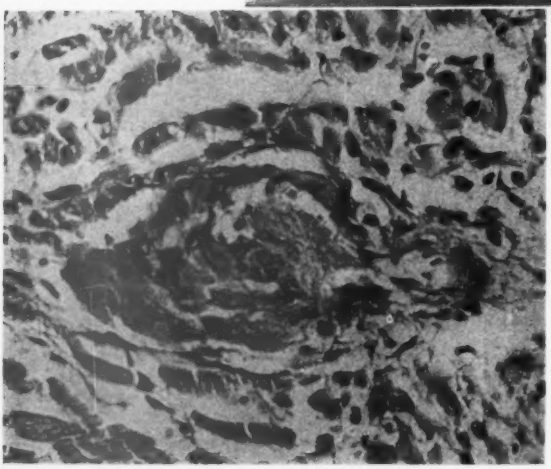

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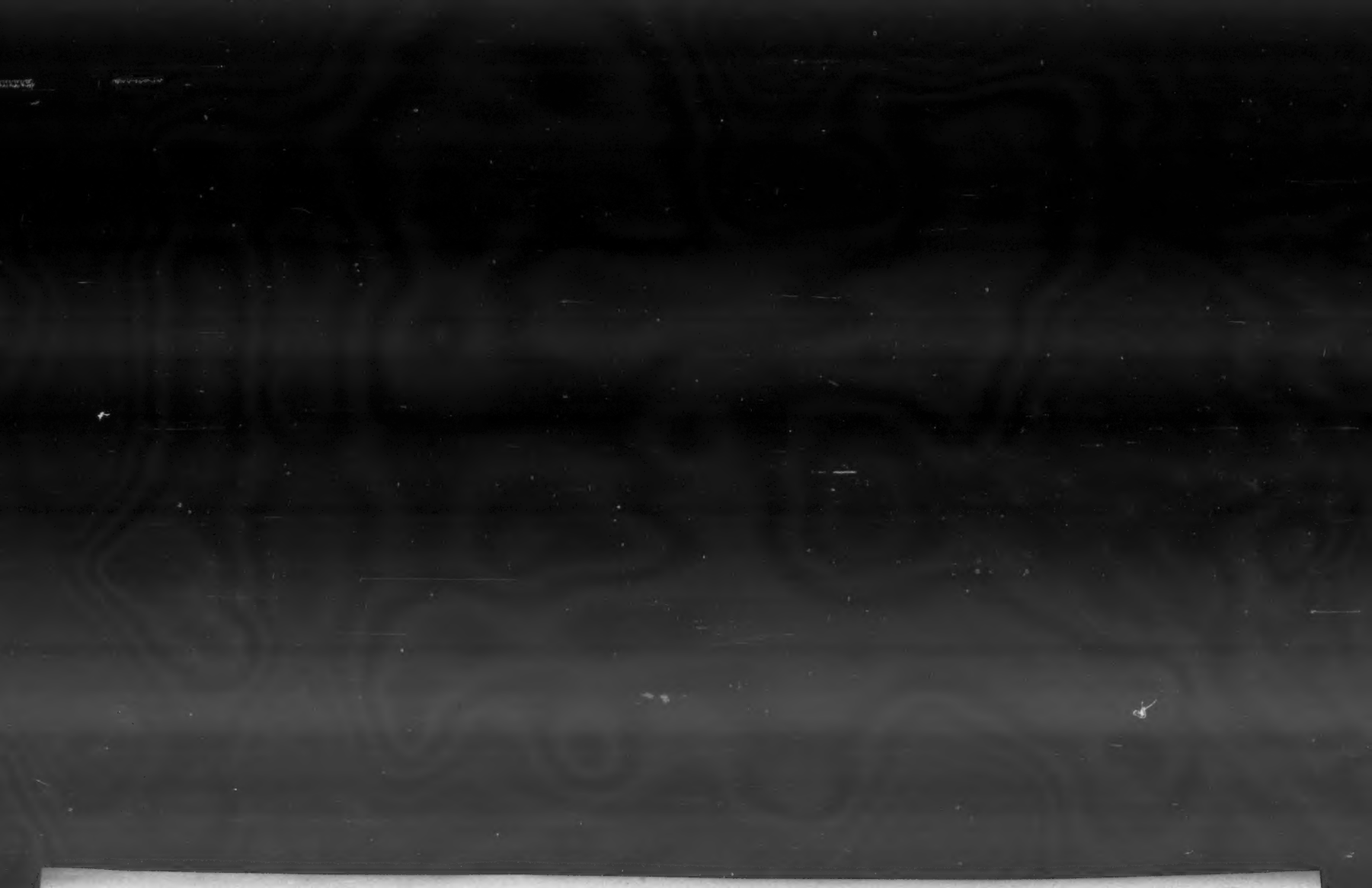
PLATE 21

FIG. 8. Photograph of the completed clay model representing a central artery with two of its branches. From each branch arise numerous capillaries distorted by aneurysms near their origin from the arteriole.

FIG. 9. Photomicrograph of two aneurysms filled by proliferated endothelium. × 312.

FIG. 10. Photomicrograph of an aneurysm filled almost entirely by typical amorphous and granular material. × 312.



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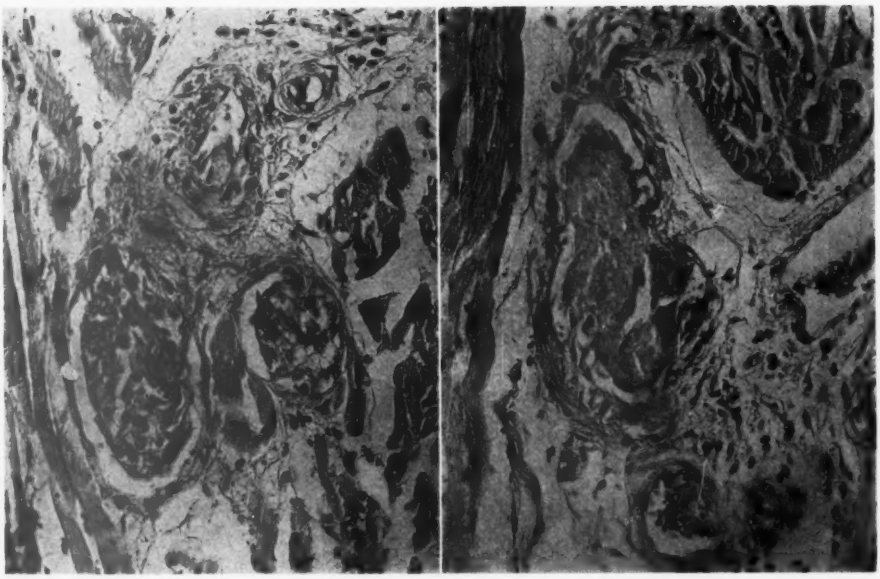

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Orbison Thrombotic Thrombocytopenic Purpura

PLATE 22

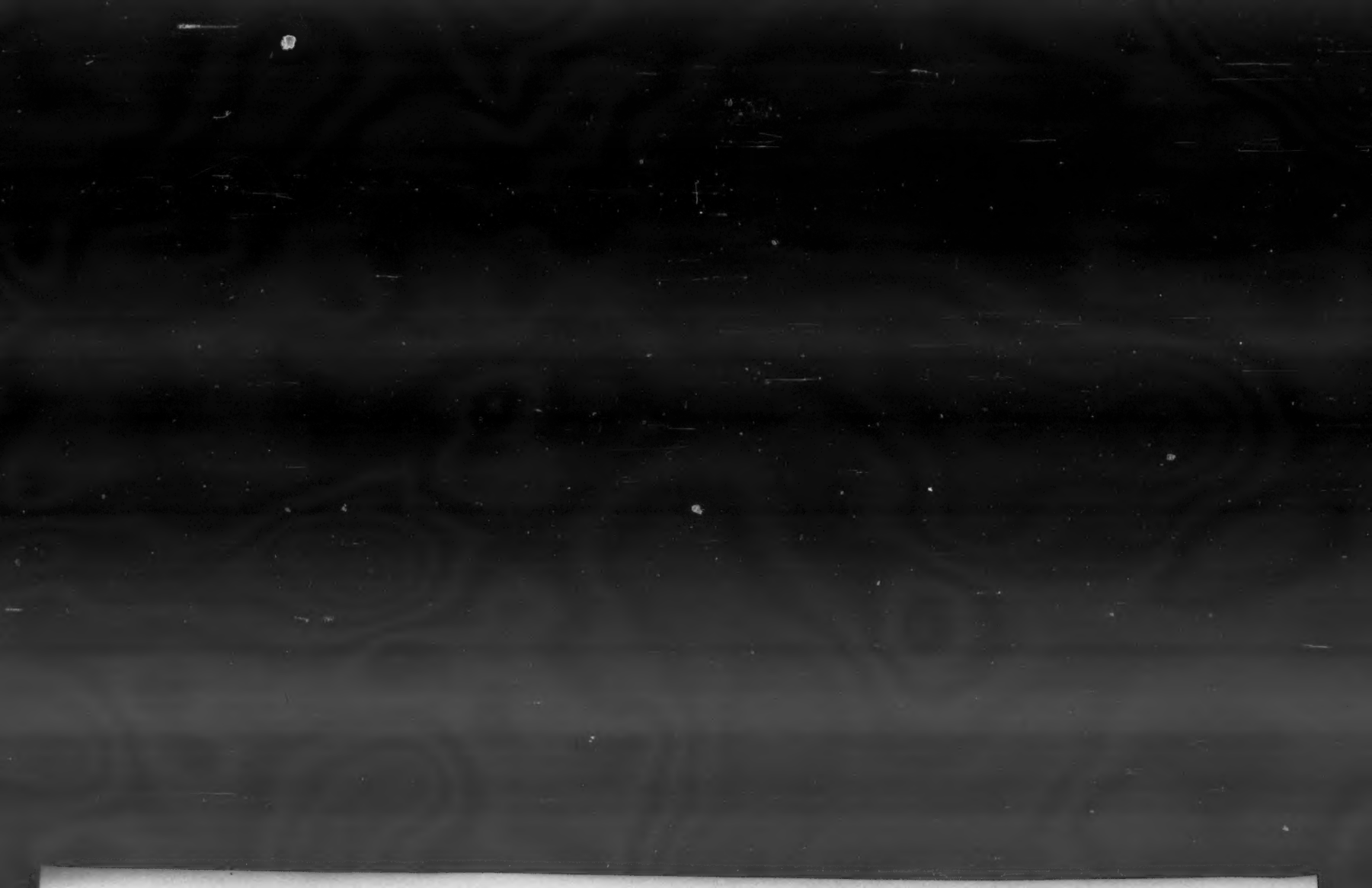
FIG. 11. Lateral view of the right arterial branch seen in Figure 8.

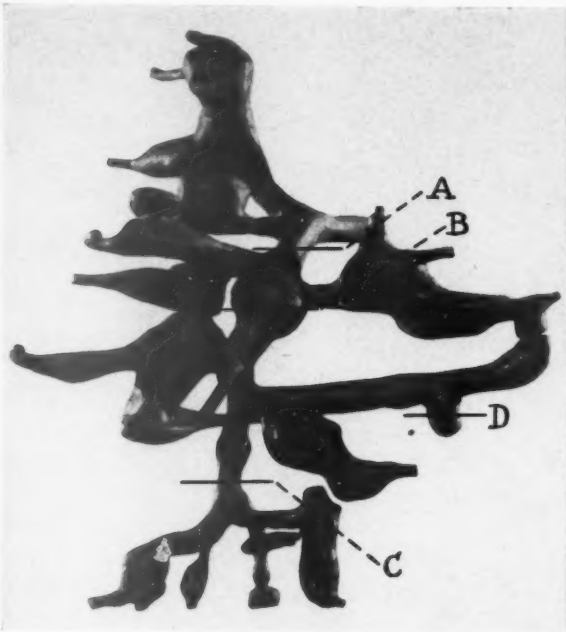
FIG. 12. Photomicrograph of the artery at the level of line A in Figure 11. × 312.

FIG. 13. Photomicrograph of the aneurysm at the level of line B in Figure 11. × 312.

FIG. 14. Photomicrograph of the artery at the level of line C in Figure 11. × 312.

FIG. 15. Photomicrograph of the aneurysm at the level of line D in Figure 11, to illustrate the one hemorrhage identified in this reconstruction and to show the amorphous, eosinophilic character of the wall of the aneurysm. × 312.





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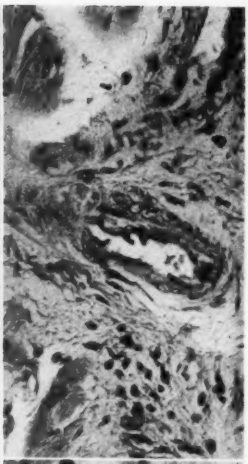

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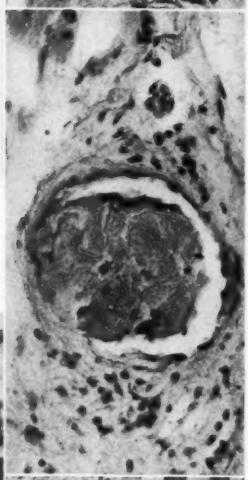

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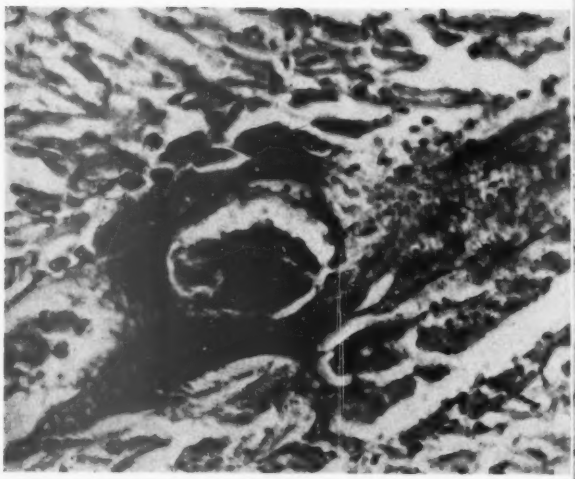

15

DESTRUCTIVE LESION OF THE ADRENAL GLAND IN SOUTH AMERICAN BLASTOMYCOSIS (LUTZ' DISEASE)*

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It is generally accepted that in typical Addison's disease, the destructive lesion of the suprarenal glands must be of a chronic progressive nature such as might be associated with amyloidosis, tumor formation, and tuberculosis. However, modern statistics suggest that atrophy and aplasia of the cortex account for 50 per cent of the cases.

In the extensive literature on blastomycosis caused by *Paracoccidioides brasiliensis* or Lutz' disease (Fonseca and Leão,[1] Almeida,[2-4] Almeida, Lacaz, and Cunha,[5] Lima,[6] Motta and Pupo,[7] Moore,[8] Motta,[9-11] Niño,[12] Fonseca,[13,14] Fialho,[15] Versiani and Bogliolo[16]), lesions in the adrenal glands were seldom mentioned. Azevedo[17] was the first to report extensive necrotic changes in one case and brief references to the subject were made afterwards by Chirife,[18] Prado,[19] and by Fialho.[15]

In 2 patients necropsied at the Division of Pathology of this Institute, the lesions in the suprarenal glands were marked and comparable in extent to those reported as due to the tubercle bacillus in cases of Addison's disease. As no reference to the syndrome of adrenal insufficiency is made in the literature on South American blastomycosis, we present our findings which are confirmative of those of Azevedo[17] in order to stimulate further investigations on the functional significance of such changes.

New data were obtained in connection with the cause of the necrosis so often found in lesions produced by *P. brasiliensis*.

Report of Cases

Case 1

A. B. was a Spanish white male, 58 years old, measuring 172 cm. in height and weighing 40 kg., who had lived in Arial, Estado do Rio de Janeiro, Brazil.

Anatomical Diagnosis (necropsy no. 8038). Blastomycotic ulcerative lesions in the plantar region of the right foot, the nostrils, and nasal septum; blastomycosis of two superficial cervical nodes (right side); blastomycotic bronchopneumonia and micro-abscesses in both lungs; bronchiectasis; fibrosis of the lungs (extensive); caseation of

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both adrenal glands; edema and chronic passive hyperemia of the lungs; acute peribronchial lymphadenitis; dilatation of the heart; bilateral hydrothorax (slight); chronic passive congestion of the liver and kidneys; arteriosclerosis of the aorta (marked) and coronary vessels (slight); atrophy and fibrosis of the spleen (weight, 50 gm.).

The adrenal glands were moderately enlarged, the left weighing 12.75 gm. and the right, 9 gm. This enlargement was better seen in transverse sections which measured 21 by 10 mm. in the wider portions of the left adrenal gland. The cut surface showed several irregular, opaque, yellowish nodules, the largest measuring 4 by 3 mm., but most of them smaller (1 to 2 mm. in diameter).

Laboratory Findings. P. brasiliensis was isolated from a cervical lymph node by Dr. Arêa Leão of the Department of Mycology of this Institute. In sections from lungs, lymph nodes, nostrils, and adrenal glands, numerous organisms were found within the lesions, some of them showing the characteristic multiple budding.

Case 2

S. S. was an undernourished Italian, 49 years old, who had lived in Campo Grande, Distrito Federal, Brazil.

Anatomical Diagnosis (necropsy no. 7978). Blastomycotic ulcerative lesions in the tongue; blastomycosis of the left superficial cervical, left submaxillary, mediastinal, and bronchial nodes; blastomycotic caseation of the left adrenal gland; blastomycosis of the spleen, kidneys, and prostate; fibrosis of the lungs (extensive); bilateral chronic adhesive pleuritis; dilatation of the heart; chronic passive congestion of the liver; thrombosis of the splenic artery and infarct of the spleen; cachexia.

The left adrenal gland was considerably enlarged and was in part destroyed by caseation. Only small portions of the cortex were still recognizable and showed several small, opaque, yellow nodules. The right adrenal gland was slightly enlarged and well preserved.

Laboratory Findings. Typical forms of *P. brasiliensis* were identified in the pus from a cervical lymph node by Dr. M. Goto of the Department of Mycology of this Institute. In sections from lungs, lymph nodes, kidneys, and left adrenal gland a great number of organisms were found within the lesions, some of them presenting the characteristic multiple budding (Fig. 6).

Microscopic Findings

The tissue was fixed in 10 per cent formalin and Helly's fluid and stained with hematoxylin and eosin, Masson's trichrome stain for con-

nective tissue, Masson's erythrosin-orange-toluidin blue stain, Verhoeff's stain for elastic tissue, and Perdrau's and Wilder's methods for reticulum.

In case 1 marked inflammatory changes and numerous organisms (*P. brasiliensis*) were found in the parenchyma of both adrenal glands as well as in the fibrous capsule and adjoining fat tissue.

The paracoccidioidal granuloma showed the usual characteristics accurately described by previous authors (Motta,[7,9-11] Niño,[12] Fialho[15]). The cellular reaction consisted of an accumulation of epithelioid cells, plasma cells, lymphocytes, a few polymorphonuclear neutrophils, and multinucleated giant cells which might contain the double contoured cell of Paracoccidioides. Proliferation of the surrounding connective tissue cells was seen and frequently the newformed connective tissue formed a wall around the lesion. Considerable destruction of the adrenal parenchyma resulted not only from the above-mentioned inflammatory lesions and fibrosis but chiefly from the associated necrosis (Fig. 1). In the necrotic areas tissue landmarks could not be made out as is the case in the lesions produced by the tubercle bacillus. However, small groups of large fungus cells, reduced to their unstained membrane (dead cells), were preserved within the caseous material. Extensive fibrosis existed around the caseous areas, and sections stained by the Masson method for collagen and by the Perdrau and Wilder methods for reticulum showed a meshwork predominantly formed by collagen fibers. No tubercles or tubercle-like foci were found around the region of caseation. Marked infiltration of the medulla by plasma cells and lymphocytes was found in some preparations (Figs. 2 and 3).

In some small blood vessels the lumen was completely blocked by large yeast-like bodies (Figs. 2 and 3). Multiplication of the nuclei of the endothelium and formation of giant cells from them were the immediate consequences of such embolism. Infiltration of the vessel walls by plasma cells and lymphocytes also was apparent (Fig. 3). Proliferation of the endothelium and narrowing of the lumen (endovascularitis) were observed in nearby segments of the same vessels.

In later stages the destruction of tissue landmarks rendered very difficult, if not impossible, the appreciation of the vascular changes described for earlier stages. Moreover, the small caliber of the vessels and the early formation of giant cells originating from the endothelium, very soon assuming the structure of foreign body giant cells, are other factors which contribute to the difficulty. This explains why they generally escape notice.

In some slides the fibrosis was very marked and the recognition of

the organ impossible (Fig. 7). Extensive loss of adrenal tissue, especially of the cortex, was the ultimate result of the changes mentioned. In case 2 the tissue of the left adrenal gland was almost completely replaced by the paracoccidioidal granuloma rich in organisms or destroyed by caseation. Only a few cell cords of the zona fasciculata were still preserved. As in case 1, embolism of the small blood vessels by large fungus cells and endovasculitis (Fig. 6) could be detected in the vicinity of the caseation. The fibrous capsule and adjoining fat tissue were invaded by the granuloma.

Tiny miliary tubercles, indistinguishable from those found in tuberculosis and like those mentioned by previous authors as occurring in South American blastomycosis, were seen associated with marked fibrosis (partially healed lesions) in the lungs and in the spleen. At this stage, *P. brasiliensis* generally was absent in the tissue, while very numerous organisms were found in earlier stages in the same lung. On the other hand, no tuberculous changes could be identified microscopically in the lymph nodes, and this renders the diagnosis of tuberculosis very improbable. The lymph nodes showed typical paracoccidioidal granuloma and a great number of organisms as usual. Paracoccidioidal granuloma and micro-abscesses containing numerous organisms were demonstrated also in the medulla of the kidney and pelvis.

Comment

It is generally recognized that necrosis is dependent chiefly on local ischemia or toxic injury, or both. In the changes here reported it is difficult to decide which of the factors is at work. Certainly local ischemia does exist as the result of the large number of organisms (*P. brasiliensis*) accumulated in the tissue and the associated inflammatory changes. Very likely this is the chief factor in the production of caseation of the adrenal gland and the condition may be aggravated by embolism of the small blood vessels by large fungus cells and by endovasculitis. Supposed toxic agents developed by *P. brasiliensis*, however, are accepted by most authorities as the cause of necrosis in the paracoccidioidal granuloma. It is impossible to prove or to disprove such a view on histopathologic grounds alone, but we were impressed by the total absence of similar necrotizing properties on the part of the fungus in other tissues. We refer to the fields in which organisms were found in enormous numbers while the adjoining cells of the tissue and the exudate were well preserved. This is well illustrated in the literature,[20] and was seen also in our preparations.

P. brasiliensis often is found in the circulating blood, as Pereira and Vianna,[21] Montenegro,[22] Rosenfeld,[23] and Madeira, Lacaz, and Forat-

tini[24] have demonstrated by hemoculture and by direct observation after centrifugation. The occlusion of small blood vessels by large organisms, therefore, is liable to occur and would explain the irregular distribution of lesions in such organs as the lymph nodes, which is so puzzling to the pathologist.

A similar result of the cutting off of the blood supply is observed in syphilis, but the vascular changes are entirely different in that disease. In syphilis there is primarily an infection of the blood vessels, chiefly the arteries, and the consequent thickening of their walls frequently leads to partial or complete occlusion of the lumen. In Lutz' disease there is at first mechanical obstruction of the lumen of small blood vessels by large forms of the fungus, followed by proliferation of the endothelium, formation of multinucleated giant cells engulfing the organisms, and endovasculitis in the neighboring segments of the vessel—changes which very likely determine the onset of the paracoccidioidal granuloma. Such vascular changes (endovasculitis) were of common occurrence in the cases studied by Fialho.[15] He made no reference, however, to embolism of the small blood vessels by fungus cells.

It is reasonable to assume that the structure of the adrenal gland facilitates the appreciation of the vascular changes here reported, as it is considered one of the most vascular organs in the body. Bailey's[25] Figure 407 gives a good idea of the richness of the blood supply of the adrenal gland of the dog.

Summary

In 2 cases of South American blastomycosis (Lutz' disease) with extensive destructive lesions of the adrenal gland, embolism of the small blood vessels by large fungus cells, giant cells derived from the vascular endothelium with many organisms (*Paracoccidioides brasiliensis*), and endovasculitis were frequently found in the paracoccidioidal granuloma.

The caseation necrosis, responsible for considerable loss of glandular tissue, is apparently a consequence of local ischemia as a result of embolism by large organisms with endovasculitis of the small blood vessels and the associated granulomas.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

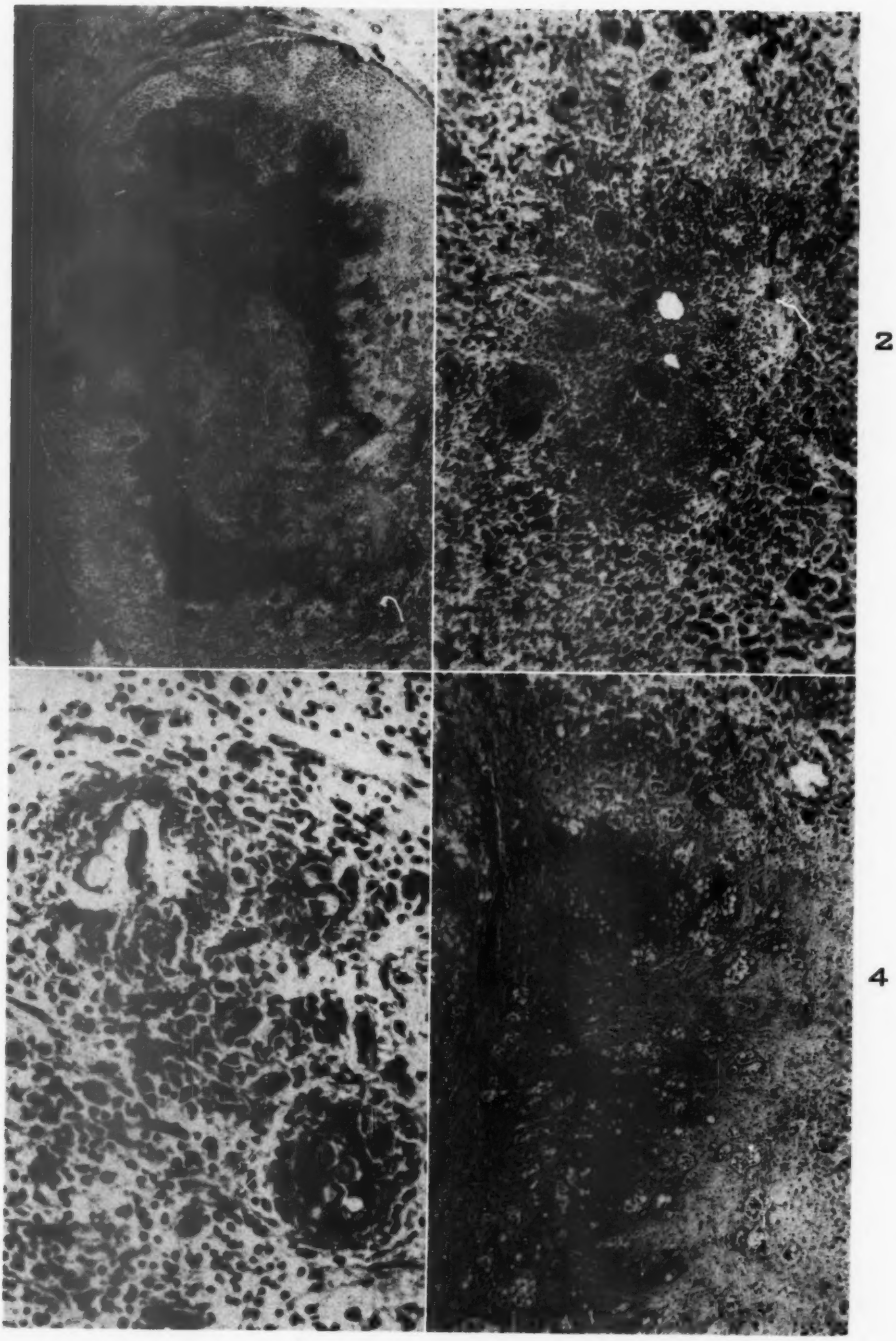
PLATE 23

FIG. 1. Case 1. Extensive caseation of the left adrenal gland. Numerous large fungus cells (*Paracoccidioides brasiliensis*) are found within the necrotic area. Hematoxylin and eosin stain. × 7.

FIG. 2. Case 1. Left adrenal gland showing infiltration of the tissue by plasma cells and lymphocytes. Small foci of necrosis. Hematoxylin and eosin stain. × 35.

FIG. 3. Case 1. Higher magnification of the small blood vessel represented in Figure 2, of which the lumen is filled by organisms (*P. brasiliensis*). Proliferation of nuclei in endothelium. Hematoxylin and eosin stain. × 170.

FIG. 4. Case 1. Caseation of cortical layer (left adrenal gland). Giant cells containing organisms (*P. brasiliensis*) are found within and about the necrotic areas. Paracoccidioidal granuloma invades the fibrous capsule and adjoining fat tissue. Hematoxylin and eosin stain. × 35.



Magarinos Torres *et al.* Adrenal Gland in Blastomycosis

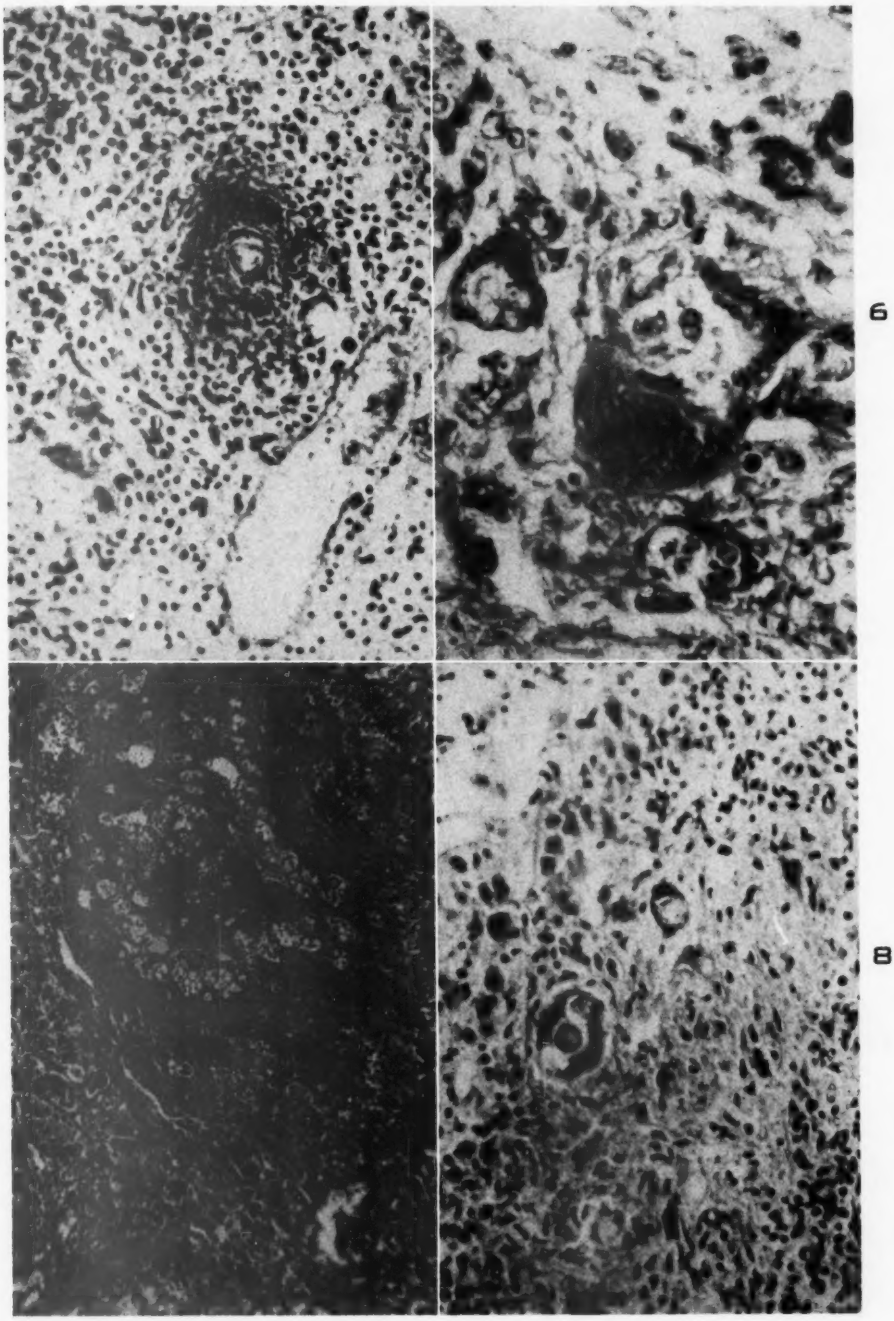
PLATE 24

FIG. 5. Case 1. Paracoccidioidal granuloma in the left adrenal gland. Beginning necrosis around a small blood vessel which is blocked by a large organism (*P. brasiliensis*) and shows proliferation of the endothelium. Hematoxylin and eosin stain. × 170.

FIG. 6. Case 2. Paracoccidioidal granuloma in the left adrenal gland containing many organisms. Proliferation of nuclei from endothelium, formation of multinucleated giant cell, and narrowing of the lumen of a small blood vessel (endovasculitis). Masson's erythrosin-orange-toluidin blue stain. × 380.

FIG. 7. Case 1. Left adrenal gland. Fibrosis and cellular infiltration around caseous necrotic material in the zona fasciculata. Masson's trichrome stain for connective tissue. × 35.

FIG. 8. Case 1. Cortical layer of the left adrenal gland. Infiltration by lymphocytes and plasma cells, fibrosis, and embolism of two small blood vessels by organisms (*P. brasiliensis*). Hematoxylin and eosin stain. × 170.



Magarinos Torres *et al.* Adrenal Gland in Blastomycosis

DEMONSTRATION OF ACID-FAST BACILLI IN TISSUE SECTIONS*

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I

Procedures for Paraffin Sections

Satisfactory demonstration of acid-fast bacilli in paraffin sections is not easily accomplished. Whether one is studying their distribution in a frankly positive lesion or searching for them where they are few, the objective is not merely to reveal some of those present but all of them as nearly as possible, the old and deteriorated organisms as well as the relatively invulnerable young forms. The difficulty is a general one,[1,2] although it is more serious with the relatively labile leprosy bacillus than with that of tuberculosis. An adequate explanation of this difficulty has not been seen.

The trouble is due to the liability to extraction of the waxy complex upon which acid-fastness depends, in the first place by the sequence of reagents involved in the imbedding and deparaffinizing process, or later, if the bacilli have survived to that point, by the final dehydrating and clearing sequence. The principle involved may be stated as follows: When acid-fast bacilli are exposed to a reagent which affects the integrity of the waxy complex but does not of itself extract it, that complex may nevertheless be "conditioned" in some way so that on subsequent exposure to another such reagent, which likewise, by itself, would not cause extraction upon primary exposure, many if not most of the bacilli are rendered unstainable. This sequence constitutes, to give it a name, the double jeopardy condition. The reagents involved need not both be active lipid solvents; one, for example, may be the cedar oil used for clearing the tissue before paraffin, the other the xylene used to deparaffinize the sections.

For one example of this effect, smears or sections in which the bacilli have faded cannot be restored by cleaning with xylene, passing to water through alcohol, and restaining; few, if any, will then retain the stain. Again, if the bacilli are suspended in chloroform, as in the Dharmendra method[3] of preparing lepromin, their acid-fastness is not lessened; but when, after the chloroform has been evaporated, the residue is taken up in ether to permit separating the bacillary bodies by centrifuging, few of them remain acid-fast or even distinguishable as blue-staining ghost forms.

Two principles are involved in efforts to overcome this difficulty with

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sections before staining. One is the protection from extraction of the conditioned and vulnerable bacilli while removing the paraffin. The other is the restoration of the acid-fastness of bacilli which have been extracted in the deparaffinizing process. Most difficult to accomplish, apparently, is the demonstration of bacilli which have degenerated and become non-acid-fast in the lesion, or in the paraffin block during storage.

Applications of the Principle of Protection

In the earlier days of the study of the histopathology of leprosy, some workers avoided the harmful effects of the dehydrating-clearing sequence by drying the sections before mounting the coverglass. The protection principle was applied in a unique manner in a staining method which I devised 25 years ago. The technic was never published, because I was never fully satisfied with it, but it has been used continuously in this institution and exclusively until the advent of Fite's new-fuchsin-formaldehyde method,[4] which stains the bacilli deep blue.

The essential feature of this method is that the paraffin is removed at the outset, and the water at the end, by essential oils which exert minimal effects on the bacilli. To deparaffinize, the slides are immersed in a suitable oil for twice as long as is necessary to remove the visible wax from around the sections. Origanum or bergamot oil usually has been used, but cedar oil is better for tissues which have been subjected to that oil in imbedding. As much of the oil as possible is blotted away and the sections are washed in running water for 1 or 2 hours, after which they are stained, decolorized, and counterstained in the usual way. The sections are then blotted until dull-dry and covered with a few drops of anise oil. By pouring this off, blotting again, and replacing the oil two or three times the sections will clear without shrinkage, ready for mounting the coverslip.

Applications of the Principle of Restoration

The principle of restoration of acid-fastness of bacilli which have been damaged by reagents may be illustrated by two simple situations from recent experience. The defatted leprosy bacilli in the Dharmendra[3] lepromin suspension can be made fully acid-fast again if the ether solution of the lipids from the bacilli and tissue is emulsified in the suspension and the ether boiled off.[5] With a suspension of bacilli which had been more or less damaged by the concentration method described by Henderson,[6] restoration has been effected in smears by treating them with paraffin oil (liquid petrolatum), of which more is to be said.

Faraco's Method. The restoration principle was introduced in 1938

by Faraco,[2] who applied it first to defatted tubercle bacilli in sputum and then to leprosy bacilli in sections. The xylene used to remove the paraffin is replaced with several drops of olive oil or a mineral lubricating oil and the slide is heated for some minutes, after which the sections are blotted to opacity and placed in carbol-fuchsin. This procedure, as well as the after-treatment, is cumbersome and not clean, nor is it always successful.*

Fite's 1947 Method. A procedure avowedly suggested by that of Faraco, and designated the Fite-Faraco method by Lillie,[7] was introduced in 1947 by Fite, Cambre, and Turner.[8] In this method the paraffin is removed by a mixture of two parts of xylene and one part of cottonseed oil,† applied for 2 to 4 minutes in two changes. The sections are blotted to opacity and stained for 15 to 30 minutes without heating, decolorized with 1 per cent hydrochloric acid in 70 per cent alcohol, and counterstained with methylene blue. The sections are then dried before mounting.

The use of the paraffin solvent in combination with an oil constitutes a most important new maneuver in dealing with the problem, effective not only at the stage of removing the wax but also at that of preparing the section for mounting the coverglass, for the oil retained by the tissue protects it from excessive shrinkage. In my experience this technic has proved very good with fresh tissues from active lesions but it has not been all that was desired when working with difficult material, and in neither case will it demonstrate all of the bacilli that can be stained.

A Modification of Fite's Method

Recent attempts to arrive at an improved method were based on the observation that high-test gasoline is more sparing of "decrepit" bacilli than most other solvents, and on the restorative effect of paraffin oil for damaged bacilli. Other materials tested with smears of such bacilli, and, in part, with sections, comprised chicken, hog, and human fat, and the total lipids extracted from leproma tissue and bacilli by the chloroform and ether sequence, all of them applied both hot and cold; coconut, olive, and cottonseed oils; and several essential oils. The best of them was much inferior to paraffin oil.

In attempting to apply this material in a simple and practicable method for staining sections, it was for a time used alone to dissolve the paraffin wax from them. No thoroughly satisfactory and dependable

* Some Brazilian workers prefer chicken fat, which Faraco also mentioned. In a demonstration with that material given me by an experienced technician in São Paulo the bacilli failed to stain.

† Peanut and olive oils are mentioned as alternatives, and it is stated that almost any non-volatile oil will serve the purpose, including liquid petrolatum.

procedure was arrived at, however, until the oil and gasoline were employed in combination, as in Fite's method. Later it was found that rectified turpentine is at times, but not always, in some ways better than gasoline.

Procedure

1. Deparaffinize the sections by immersion for 1 or 2 minutes in a 1:2 mixture of paraffin oil (either light or heavy grade) and high-test gasoline (aviation grade without lead; color of no consequence), or of the oil and rectified turpentine. If multiple slides are to be stained, they may be immersed and removed in turn at 1-minute intervals, or, with practice in the next step, at 30-second intervals.
2. Blot to opacity and place in water until the series is ready.
3. Stain in carbol-fuchsin at room temperature, 15 to 30 minutes (20 minutes being ample in summer weather). Wash.
4. Decolorize the sections one by one until they, or at least the portions not loaded with old fuchsin-retaining foamy cells, are of the proper pink color. For regular use, 20 per cent aqueous sulfuric acid is preferred, but if the tissue retains too much of the dye, 2 per cent hydrochloric acid in 70 per cent alcohol may be better. Place in water until all are ready for counterstaining.
5. Counterstain lightly in Loeffler's methylene blue, one-fourth or one-eighth strength. Wash.
6. From the water, wipe the slides clean, and allow the sections to dry in the air.
7. Mount the coverglass with a good synthetic mounting resin.

Special Restorative Methods

Although Fite's recent method has been regarded as a development of that of Faraco, it is actually a protective rather than a restorative one. The short period of application of his solvent-oil mixture is insufficient to restore acid-fastness to bacilli which have been extracted, and no such effect has been obtained by prolonging the exposure; indeed, the bacilli are sometimes rendered thinner and paler. The modification of his method given above is similarly a protective procedure. Although the regular method given will ordinarily demonstrate more acid-fast forms than any other simple one, still more can be revealed in most specimens by special treatment with paraffin oil.

For this purpose a heavy grade of paraffin oil, some four times as viscous as the light grade, is somewhat the better. No advantage has been found in heavier oils such as those mentioned by Faraco, nor in the application of heat up to 100° C.

Prolonged Treatment of Ordinary Slides. Sections mounted on slides in the usual manner are immersed at room temperature in the gasoline-oil or the turpentine-oil mixture, or in pure paraffin oil, for 2 to 3 hours (extremes, 1 to 6 hours). Thereafter they are blotted and processed as described, preferably using acid alcohol after the gasoline-oil mixture, and aqueous sulfuric acid after the turpentine-oil preparation.

Mounting Sections with Oily Materials. For this purpose it is necessary to use slides on which a thin layer of the egg albumin mixture previously has been dried. Several drops of the gasoline-oil or the turpentine-oil mixture are spread on the slide so prepared and the paraffin ribbon is laid on it. The wax dissolves slowly and wrinkles straighten out if enough of the solvent fluid is used. After the wax has dissolved, the fluid is first wiped* and then drained off. The sections are then treated with a little more of the mixture, to ensure complete removal of the wax, and, after draining is complete, they are blotted. The slides may be dried in the paraffin oven for 2 or 3 hours or longer at lower temperatures, and are stained on the same day. It is well to blot them again after drying, to ensure affixation.

Pure paraffin oil may be used, but it dissolves the wax much more slowly and often incompletely, and the sections do not position as readily. Because the gasoline evaporates rapidly, the mixture with that solvent is in effect equivalent to pure oil in a more practicable form.

The first of these restorative methods will commonly reveal more bacilli than the regular technic, and often many more. This holds true not only for old foamy and other lepra cells in which there obviously has been much deterioration, but also for those of younger lesions which contain numerous invulnerable bacilli. The second method has often given maximum demonstration of bacillary forms, and at times the results have been extraordinary. There is, however, a disadvantage in that the clusters of bacilli often show an effect of loosening and spreading, so that they are not as well confined to their original locations as when the first method is used. After either method the intracellular clusters of bacilli often are found to be arranged in radiating, stellate fashion instead of being in the more familiar packets, a condition noted by Leon-Blanco and Fite[9] after impregnation with silver.

Comments

Of primary importance in work with the leprosy bacillus, is the fact that no single procedure can be expected to give the best results in all cases. There are too many variables with respect to the condition of

* For wiping, facial tissue is most practicable.

the bacilli and the nature and condition of the cells which contain them.

In the macrophage type of leproma the cytoplasm of the older lepra cells, the familiar Virchow foamy cells, is so tinctured by the products of the bacilli contained in it that it tends rather strongly to retain the fuchsin. On the other hand the elongated cells of the histiocyte type of leproma, which are typically crowded with nude bacilli without globar material and which consequently have relatively little tendency to vacuolation even when old, are much less retentive of the dye; and that holds true for the epithelioid cells of the tuberculoid lesion of leprosy.

In the lesions of an untreated or unsuccessfully treated case, the lepra cells of whatever type usually are crowded with bacillary forms of ordinary appearance. In treated cases which have improved, especially in these days of sulfone therapy, the bacilli may be relatively few, and when stained they may be smaller, thinner, and paler, or appear only as granules. In either case some of the bacilli are relatively resistant to adverse conditions and are both acid-fast and alcohol-fast; others retain the dye against acid but not alcohol, while still others are non-resistant and may not be demonstrable except after special restorative treatment. If, after the usual procedure, there is good demonstration of bacilli in a young area of an active lesion, one should not conclude that the few which are seen in older, foamy-cell areas are the only ones present. Large numbers of tinctorially degenerated but undigested bacillary forms may actually be present, illustrating the poor capacity of these cells to destroy bacilli completely.

With regard to fixation, Mallory[10] recommended Zenker's fluid for tissues in which bacteria are to be demonstrated, and Leon-Blanco and Fite[11] recently have shown it to be best for the leprosy bacillus. The tissues should be thoroughly washed after fixation; otherwise the tissue elements may hold the fuchsin excessively. After-treatment with alcohol should not be shortened. Treatment of the tissues or sections with iodine is not necessary, nor in fact desirable.

The over-all effects of the two solvent-oil mixtures specified differ materially in certain respects. After the gasoline mixture the foamy cell areas hold the fuchsin more strongly than after the turpentine mixture. Often this is beneficial, but if too much is held the bacilli are not properly differentiated. With a similar mixture of petroleum ether and paraffin oil this tendency usually is greater, for that solvent is even less active in extracting from the cells the element which makes them acid-resistant. In either case the bacilli are typically a clear, brilliant red.

After the turpentine-oil mixture the sections decolorize more quickly and much more uniformly, although in the regular method the foamy

cells usually retain some red, and the counterstain is taken more deeply. After prolonged exposure, the general effects are the opposite of those imparted by the hydrocarbon solvents. The connective tissue, especially, tends to retain the fuchsin, while the foamy cells may give it up and take the counterstain; yet with proper treatment the bacilli in them remain well stained and in correspondingly strong contrast. With either procedure, the bacilli after turpentine are darker and duller, which may or may not be an advantage.

Any usual carbol-fuchsin may be employed. For the leprosy bacillus, 20 minutes at ordinary room temperatures is ample; for the tubercle bacillus, 1 hour in the paraffin oven is needed, or proportionately longer at a lower temperature. In neither case is undue prolongation of the time beneficial, and after a certain point the tissue elements retain too much of the dye.

No one decolorizer should be depended upon exclusively. Ordinarily, acid alcohol decolorizes the sections more uniformly than do the aqueous acid solutions. This is often an advantage with respect to contrast, but not with respect to the demonstration of the greatest possible number of bacillary bodies unless the foamy cells are retentive of the fuchsin, as after the restorative procedures. Aqueous sulfuric acid has less effect on these cells; nitric acid has the least effect, and has little to recommend it.

Contrary to a prevalent idea, the sections should not be exposed to the decolorizer longer than necessary, and one must often choose whether to treat for the more readily decolorized areas of a section or the resistant ones. Furthermore, the treatment should be active, the slides being sloshed back and forth in a dish of the reagent and not merely immersed in it. After the turpentine-oil mixture the time required is usually less than 15 seconds, and after the gasoline-oil mixture it is seldom more than 20 seconds. Stop-watch control is desirable to ensure uniform treatment of a batch of slides.

The expedient of drying the sections before mounting is permissible because the tissue elements retain enough of the oil through the staining process to give much protection from shrinkage. This can readily be demonstrated by including in a set of slides some that have been deparaffinized in the usual way.

With regard to the medium for mounting coverslips, it is difficult to choose between permount* and the H.S.R.† product. Certain others which have been tried seem not to preserve the stained bacilli as well.

Study of sections processed in the ways described will give to many a

* Obtained from the Fisher Scientific Co., New York, N.Y.
† Hartman Leddon Co., Philadelphia.

new idea of the tremendous numbers of bacilli, of various degrees of vitality, that are present in the full-blown lepromatous lesion, and a new appreciation of the task faced by the clinician when he undertakes by therapy to eliminate them from the patient.

Summary

The difficulty encountered in the adequate demonstration of acid-fast bacilli in sections is ascribed to extraction by serial treatment with reagents, wherein one of them affects the integrity of the waxy component upon which acid-fastness depends and the next one removes it.

The difficulty can be overcome in various degrees, before staining, by methods which either protect the "conditioned" bacilli from extraction or restore acid-fastness after that has occurred. The protective method devised by me and used in this institution for many years, based on the use of essential oils for removing the paraffin and for dehydrating and clearing after staining, is given in summary. The restorative principle, as introduced by Faraco, involves treatment of the sections with an oil after deparaffinizing.

The revolutionary method recently devised by Fite, in which paraffin is removed by a mixture of xylene and cottonseed or other similar oil, was derived from that of Faraco but is actually a protective and not a restorative one. An improvement of that method employs mixtures of paraffin oil (liquid petrolatum) and aviation gasoline or rectified turpentine, the two preparations giving somewhat different effects.

This improved method is also essentially of a protective nature. However, by treating sections mounted in the ordinary way for some hours with the mixtures specified, or by mounting sections on dried albuminized slides with those mixtures, a considerable degree of restoration can be obtained, and the results are sometimes remarkable.

No single procedure or schedule can be expected to give the optimum results with all specimens, and hence the various factors are considered in some detail.

II

Application of the Carbowax Technic

To avoid the harmful effects, on acid-fast bacilli in tissues, of the sequence of reagents involved in paraffin imbedding, a matter discussed in part I of this article, some workers have resorted to frozen sections, with or without infiltration with gelatin or agar. Good results have been obtained, but such methods are neither convenient nor entirely practical.

Recently introduced into histologic technic are certain waxy sub-

stances, polyethylene glycols called carbowax compounds,* in which tissues can be imbedded directly from water or alcohol. When it was learned that it is feasible to section tissues such as skin in carbowax mixtures, some of them were acquired for trial. They have proved entirely practicable, once the technician becomes accustomed to the characteristics of this odd medium, and eminently useful for the demonstration of leprosy bacilli. The process is protective to a considerable degree, and the sections are susceptible to the restorative measures that have been found useful for paraffin sections.

General Considerations

The carbowax method, being new and not yet standardized, presents certain problems peculiar to itself which are considered from a general point of view in another paper.[12] The different grades of carbowax, which are designated by numbers representing their average molecular weights, differ widely in hardness and in hygroscopicity. The 1000 grade is moderately firm and the 1540 grade more so, whereas the 1500 grade is of the consistency of petrolatum because it is a mixture of 1540 and a liquid polyethylene glycol; the 4000 wax is quite hard. The 1000 grade is so hygroscopic that on exposure to atmospheric moisture in this locality it becomes fluid in a day or so, while the 4000 grade has shown no such effect over a period of several months. There are indications that different lots of the same designation may differ somewhat in their hardness. An important fact is that the manner in which these materials are heated and cooled affects their mode of crystallization and their consistence.

The usual embedding mixture used[13-15] consists of 1:9 parts of the 1500 and 4000 grades, although it was said[14] that that is too soft for summer conditions in Maryland. By fortunate inadvertence, instead of the soft 1500 grade, I was supplied the firmer 1540 variety. A 1:9 mixture of it and 4000 is sometimes too hard to ribbon properly even in warm weather. A 2:8 proportion usually has been better, but a 15:85 mixture has proved best when the temperature reached 90° F. or higher. Firminger's[14] procedure of heating a newly prepared mixture briefly to 175° C. has been found at times disadvantageous. Melting should be done in the paraffin oven, and not over the open flame.

With regard to infiltration, fat is not penetrated by carbowax, hence the subcutis cannot be studied as well as in paraffin sections, and even intracellular fat, if excessive, interferes with penetration. Infiltration

* Products of Union Carbide and Carbon Corporation, 30 East 42nd St., New York 17, N.Y.

times specified vary from 1 to 3 hours, but for old leproma specimens with foci of lipid-rich foamy cells a longer period is better. Some trouble has been met with on this account in dealing with caseous lesions of tuberculosis.

There are problems in connection with the flotation of the ribbons and the affixing of the sections to the slides. No material has been found which permits the floating and stretching of carbowax ribbons intact as is done with paraffin ribbons, and apparently Mayer's egg albumin as used with such ribbons has not been found reliable for affixation. The procedure here given has proved satisfactory in extensive experimentation in this laboratory.

Procedure

Preparation of the Tissue. Blocks of Zenker-fixed and thoroughly washed tissues, cut to the usual thickness and trimmed of gross fat, may be transferred directly to the melted carbowax mixture from the wash water, as may be done from formalin if that fixative is used. It has been found better, however, to treat Zenker-fixed tissues overnight with 80 per cent alcohol, from which also transfer can be made directly to the wax.

Preparation of the Carbowax Mixture. A 15:85 mixture of the 1540 and 4000 grades is recommended. The ingredients are melted together in a beaker in the paraffin oven (56° to 58° C.), and the mixture may be kept there indefinitely.

Infiltration. Infiltrate in the melted carbowax mixture, 2 changes, for 2 to 6 hours according to the size and character of the specimen. The tissue blocks should be stirred about occasionally, for the material is viscid and does not take up water with avidity.

Blocking. This is done as usual in paper boats, in wax not hotter than the temperature of the paraffin oven. The blocks are solidified in the refrigerator, after which they are allowed to warm to room temperature before the paper is removed.

Sectioning. Preparing the blocks for sectioning is done precisely as with paraffin blocks, with care to make the upper edges exactly parallel. Cutting is done at room temperature. If the blocks are too hard to form ribbons, or if ribbons break up too badly on handling, that condition may correct itself spontaneously in a day or two. It can usually be corrected at once by "doping" the upper and lower surfaces with 25 per cent beeswax in chloroform painted on with a fine brush and allowed to become dull-dry. Plain water also may serve this purpose. The ribbons are laid out on paper or in a suitable container and cut into short lengths.

Preparation of Slides. Difficulty in affixing sections to the slide is avoided by the use of dried albuminized slides, as used by Mann.[16] A drop of Mayer's egg albumin solution is rubbed over the slide with the finger and the excess wiped off with a firm stroke or two of the side of the hand, and the slides are then dried overnight at room temperature or for 2 or 3 hours in the paraffin oven. If too much albumin is left on the slide, the glycerin prevents proper drying. There is little danger of removing too much.

Flotation of Ribbons. Because the wax dissolves immediately on all watery flotation media and on most others, leaving the sections nude, it is best to spread a few drops of the flotant on the prepared slide and then lay a suitable length of ribbon upon it. After the wax is dissolved the sections are held in position with the teasing needle (the needle held below and not on them) while the slide is drained first sideways and then obliquely. The slides are dried in the paraffin oven for 2 or 3 hours, or otherwise overnight.

The most satisfactory flotant of many which have been tried is a 10 per cent solution of carbowax 1540 in distilled water containing 0.005 per cent of turgitol 7.* This wetting agent abolishes the violent and often injurious surface tension effects of plain water. The carbowax which remains in the sections lessens the danger of shrinkage on drying, during which the sections become bone-white.

Preparation for Staining. Before applying any ordinary staining method, it is well to treat the sections with water for a few minutes to remove any residual wax. Sections to be stained for acid-fast bacilli may, alternatively, be immersed in a 2:1 mixture of aviation gasoline and paraffin oil for a few minutes, after which they are blotted to opacity and transferred to water. After this treatment the bacilli may be stained better, and little trouble results from such carbowax as may be present. Treatment of Zenker-fixed tissue for removal of iodine is neither necessary nor advisable.

Staining for Acid-Fast Bacilli. 1. Stain with carbol-fuchsin for 20 minutes at room temperature. Wash.

2. Decolorize, preferably with 20 per cent aqueous sulfuric acid. Less time is required than with paraffin sections, often no more than 5 or 6 seconds.

3. Counterstain lightly in dilute (one-fourth to one-eighth) Loeffler's methylene blue. Wash.

4. Wipe the slides clean and allow the sections to dry in the air.

5. Mount the coverslips with a synthetic resin.

* Union Carbide and Carbon Corporation.

Restorative Treatment with Paraffin Oil

As with paraffin sections, more bacilli often can be made to stain well by restorative treatment with paraffin oil, which can be done in either of two ways.

Oil Treatment of Mounted Sections. With carbowax sections the gasoline-oil mixture seems generally preferable to turpentine and oil. The slides are left in it for 2 to 3 hours, or longer, before blotting and staining.

Mounting of Sections with Oil Medium. With some specimens the optimum effect has been obtained by using the turpentine-oil or the gasoline-oil mixture for the flotant. Although the sections become translucent, the wax does not dissolve in either of these mixtures, and wrinkles do not disappear spontaneously as they do on aqueous flotants. However, the consistency of the wax is somewhat modified, so that by gentle application of the teasing needle any wrinkling present can be considerably reduced. After draining the slide for some minutes the sections are blotted to opacity and dried in the paraffin oven for 3 hours or so, or longer at a lower temperature.

Slides so prepared are best stained the same day. After another precautionary blotting, they are treated with water to remove the wax and transferred to carbol-fuchsin. Little shrinkage occurs, but there is some danger of loosening.

Comment

In most instances the simpler staining procedure with carbowax sections reveals many more bacilli than are demonstrated in paraffin sections of duplicate tissue blocks by the same staining procedure. It has happened that with specimens from cases under active sulfone treatment the results have been disappointing and no better than with paraffin sections. However, with such difficult material the restoration methods have given better results in carbowax sections than in their paraffin opposites.

In many cells of active lesions, especially cells of the elongate histiocyte type, the bacilli will be found after restorative treatment to have the radiate arrangement that can be shown also in paraffin sections (part I). The numbers that will be found in old lesions, especially after the restorative treatment, are often amazing. Old foamy cells which ordinarily are regarded as containing mostly detritus with few actual bacilli may appear simply crowded with them.

It cannot be said that the carbowax method is entirely harmless, at least for bacilli in poor condition. If, for example, carbowax sections are treated with chloroform, which has no primary denaturing effect on

fresh bacilli from lesions or those in heat-killed tissues used for making lepromin, fewer acid-fast forms will be found than in controls, indicating that some degree of conditioning for extraction has occurred. However, the carbowax process is decidedly less harmful than the paraffin sequences. Furthermore, the restorative procedures are more effective with carbowax than with paraffin sections.

Firminger[14] has pointed out that the carbowax method is admirably suited to the study of intracellular lipids, although Lillie[17] said that this medium dissolves some lipids to a considerable extent, with specific mention of those of the adrenal gland. Whether this method of sectioning will be of value in studying with fat stains the lipid elements of the leproma has yet to be determined.

Summary

The carbowax method of sectioning tissues, which involves no harmful sequence of reagents either in the imbedding process or in dewaxing the sections, has been found eminently satisfactory for the demonstration of leprosy bacilli in lesions.

The carbowax method involves certain peculiar problems and has not yet been fully standardized. Having in view the essential features, a practicable technic has been worked out. Without special treatment, carbowax sections have usually given decidedly better results with carbol-fuchsin staining than have comparable paraffin sections, and the results of the restorative procedures have been still better in comparison.

Whether or not this method will be equally superior for the demonstration of tubercle bacilli in lesions has yet to be determined. Also to be determined is the possible value of the method for the study of intracellular lipids in the lesions of leprosy.

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